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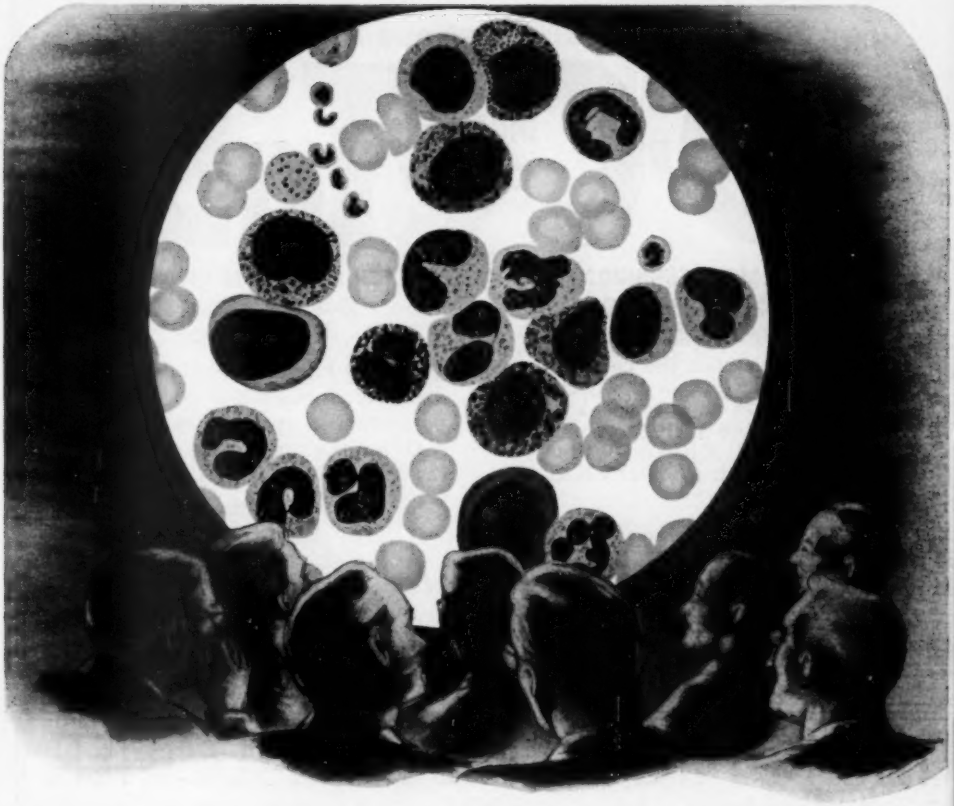

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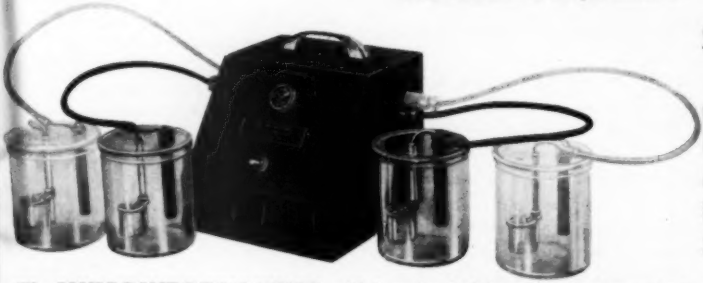

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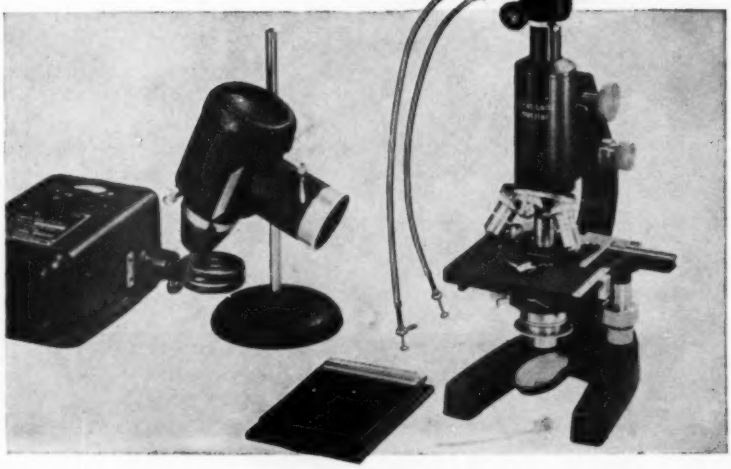

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VOLUME 51 MAY 1951 NUMBER 5

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OSTEOGENIC SARCOMA AFTER INHALATION OF BERYLLIUM OXIDE

FRANK R. DUTRA, M.D.
EDWARD J. LARGENT, A.B.
AND
JAMES L. ROTH
CINCINNATI

IT HAS been demonstrated that the inhalation of certain poorly soluble compounds of beryllium may cause granulomatous inflammation of the lungs in man. In occasional patients in whom such inflammation has developed, granulomas are also present in other organs (liver, spleen, bone marrow and lymph nodes of chest, neck, and axillas [1]).

Pulmonary fibrosis and nonspecific chronic pneumonitis may also develop in experimental animals which have been exposed daily to atmosphere contaminated with dusts of beryllium oxide or of phosphors that are in part comprised of beryllium oxide.[2] Furthermore, intravenous suspensions of these compounds administered to rabbits have induced development of osteogenic sarcomas.[3]

An osteogenic sarcoma has now developed in an albino rabbit that had been exposed repeatedly to an atmosphere containing dust of beryllium oxide; this animal had never been given an intravenous injection of any substance.

METHOD AND RESULT

In the course of experiments conducted for the purpose of reproducing in animals, if possible, chronic pulmonary berylliosis, three separate groups of animals were exposed to air containing dust of beryllium oxide for five hours a day on five days a week over periods ranging from nine to 13 months. In one experiment involving eight rabbits, the air contained the dust in an average concentration equivalent to 30 micrograms of beryllium per liter; in another involving six rabbits

This work was supported by grants from the Office of Naval Research and the United States Atomic Energy Commission.

From the Kettering Laboratory, Department of Preventive Medicine and Industrial Health, University of Cincinnati College of Medicine.

1. (*a*) Dutra, F. R.: The Pneumonitis and Granulomatosis Peculiar to Beryllium Workers, Am. J. Path. **24**:1137-1165, 1948. (*b*) DeNardi, J. M.; Van Ordstrand, H. S., and Carmody, M. G.: Chronic Pulmonary Granulomatosis: Report of 10 Cases, Am. J. Med. **7**:345-355, 1949. (*c*) Vorwald, A. J.: Pathologic Aspects of Acute Pneumonitis and Pulmonary Granulomatosis in Beryllium Workers, Occup. Med. **5**:684-689, 1948.

2. (*a*) Dutra, F. R.: Unpublished data. (*b*) Vorwald, A. J.: Animal Methods, in Vorwald, A. J.: Pneumoconiosis: Beryllium, Bauxite and Compensation, New York, Paul B. Hoeber, Inc., 1950, pp. 393-425.

3. Dutra, F. R., and Largent, E. J.: Osteosarcoma Induced by Beryllium Oxide, Am. J. Path. **26**:197-202, 1950. Hoagland, M. B.; Grier, R. S., and Hood, M. B.: Beryllium and Growth: II. Beryllium-Induced Osteogenic Sarcoma, Cancer Res. **10**:629-635, 1950.

the concentration averaged 6 micrograms of beryllium per liter of air, and in still another five rabbits were exposed to an average concentration of 1 microgram per liter of air. In addition to the rabbits mentioned above, each experiment also included exposures of several rats, mice and guinea pigs. The dust was delivered into a respiratory chamber by a device which is to be described in detail elsewhere.[3a] The diameters of the dust particles ranged from 0.11 to 1.25 microns, and the average diameter of the particles was 0.285 micron. The concentrations in the chamber were checked by continuous sampling throughout four of the five hours of exposure on each day.

The rabbit in which osteogenic sarcoma developed had been exposed 235 times, over a period of 11 months, to a concentration of beryllium oxide averaging 6 micrograms of beryllium per liter of air. The animal began to lose weight four months

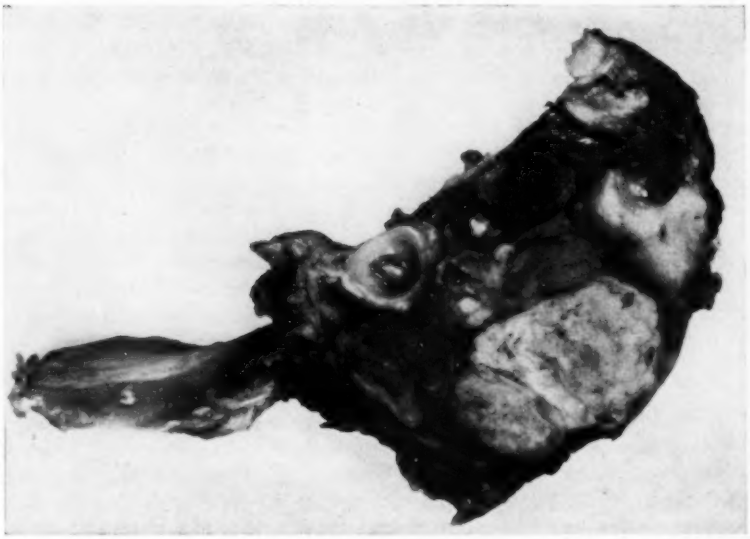

Fig. 1.—Osteogenic sarcoma extending from the inferior ramus of the left pubis into adjacent soft tissues. The acetabulum is at the approximate center of the upper edge of the specimen.

before its death, and it continued to lose weight until it died 17½ months after the last day of exposure.

The postmortem examination revealed a firm tumor arising from the medial surface of the inferior ramus of the left pubis (fig. 1). Within the pelvis was a tumor measuring 6 by 4 by 2.8 cm.; this tumor extended into the fasciae of the pelvis and beneath the peritoneum of the urinary bladder, compressing the latter but not invading the muscular portion of its wall. The tumor also compressed the lumen of the colon but did not invade the organ. The left obturator muscles were

3a. Dutra, F. R.; Largent, E. J.; Cholak, J.; Hubbard, D. M., and Roth, J. L.: Persistence of Beryllium Oxide in Lungs After Inhalation of Dust, A.M.A. Arch. Indust. Hyg., to be published.

invaded, and the portion of the tumor that extended into them showed no evidence of encapsulation. The tumor cut with moderate resistance, and the knife encountered a few small calcareous areas. The cut surface was divided into compartments by thin gray fibrous bands, and these compartments ranged from 0.6 to 2.2 cm. in diameter. The tissue in some of the compartments was homogeneous and grayish white; in others it was pinkish red and appeared slightly granular. Then again, there were regions of pale gray tissue in which there were small yellowish gray areas of ossification, while other portions were gray and glassy, appearing to be hyaline cartilage. Throughout the cut surfaces, numerous small blood vessels were visible.

Fig. 2.—Metastases of osteogenic sarcoma in right lung.

The lungs were approximately twice their expected size, and the surfaces were marked by the presence of emphysematous bullae and nodules of metastatic tumor (fig. 2). The bullae were along the sharp margins of the lobes and measured up to 1 cm. in diameter. Elsewhere, approximately one fourth of the surfaces of the lungs consisted of nodules of subpleural tumor ranging from less than a millimeter up to 1.6 cm. in diameter. Most of the masses of tumor projected above the surface of the pleura, but some were umbilicated. Where there was neither tumor nor bullae, the lobular markings were prominent because the lymphatics were engorged with grayish white material, presumably beryllium oxide. Approximately one eighth of

the total substance of the lungs was occupied by spherical masses of metastatic tumor. The cut surfaces of the tumor were for the most part white and homogeneous, but some of the larger nodules had grayish yellow calcified specks within them. Areas of hemorrhage were occasionally observed in the nodules. There was moderate enlargement of the mediastinal lymph nodes. A few spherical masses of metastatic tumor were present beneath the serosa of the parietal pericardium, the largest of these being about 2 mm. in diameter. On the anterior surface of the diaphragm were approximately 10 subpleural firm white nodules, ranging from 0.1 to 0.3 cm. in diameter. In the liver there was a single unencapsulated spherical mass of metastatic tumor that was 0.2 cm. in diameter. Eight or 10 similar nodules, ranging up to 0.8 cm. in diameter, were present in the spleen. Examination of the marrow of the long bones revealed normal marrow everywhere except in the region of the neck of the left humerus. In this area there was a well demarcated sphere of dense white tissue, 0.05 cm. in diameter.

Microscopically, the tumor was similar in all respects to the osteogenic sarcomas that follow the intravenous injection of poorly soluble compounds of beryllium. These have been described elsewhere in detail.[3] In addition to its spread in the tissues adjacent to the left pubis, microscopic examination revealed that the tumor had invaded the matrix of the bone adjacent to its site of origin. The tumor consisted of atypical, large, poorly differentiated cells, abnormal masses of hyaline cartilage and occasional poorly formed masses of osseous tissue. There were numerous large vascular spaces, many of which had incomplete endothelial linings so that they were in part lined directly by tumor cells.

Metastatic tumors which were observed grossly in the lungs, the pericardium, the diaphragm, the spleen and the liver, all reproduced cells of the primary tumor of the pubis.

Sections of marrow from various long bones revealed normal hematopoietic tissue; the sphere of dense white tissue observed in the marrow of the neck of the left humerus consisted of osteogenic sarcoma embedded in normal marrow. While this might have been a small primary tumor, it is believed more probable that it was a metastasis of the tumor of the pelvis.

In regions of the lungs that were not adjacent to nodules of metastatic tumor, there were focal areas of extensive emphysema and others of partial atelectasis. Interstitial fibrosis and lymphocytic infiltration of the alveolar walls were observed in many areas. Large amounts of highly refractile granular material were scattered in alveoli throughout the lungs; most of this was free in the alveolar spaces, but there were moderate numbers of large mononuclear cells in the alveoli, the cytoplasm of which contained debris. On the basis of examination and analysis of lungs from a number of animals that had been subjected to inhalation of beryllium oxide, these granules were believed to consist of beryllium oxide. In addition to the material within the lumens of the alveoli, large amounts of similar granular material were present in the fibrous tissue of the alveolar walls and in the peritruncal connective tissues.

There were thin zones of lymphocytes immediately beneath the capsules of the hilar lymph nodes. Large amounts of granular material were also present in the lymph nodes, so that the central portions of the nodes were almost completely replaced by large mononuclear cells filled with debris, and much debris also lay free within the tissues of the nodes.

In addition to the masses of metastatic neoplasm in the spleen, there was chronic passive hyperemia. Many large mononuclear cells which contained hemosiderin were scattered throughout the pulp of the spleen.

Tissues from the rabbit were analyzed for beryllium by the spectrographic method of Cholak and Hubbard,[4] and the results of the analyses are presented in table 1.

COMMENT

The morphology of this tumor and the distribution of its metastases indicate that it is essentially identical with the tumors that result from the intravenous injection of certain compounds of beryllium. The latter also arise in bone, tend to invade adjacent muscle and other tissues, contain large vascular spaces lined only by tumor cells and usually are productive of widespread metastases.[5]

That beryllium oxide bears an etiologic relationship to the tumor can scarcely be questioned. Such tumors have not occurred among large numbers of albino rabbits that have been used over a period of more than 20 years in this laboratory in a variety of experiments involving substances other than compounds of beryllium.

TABLE 1.—*Beryllium in Viscera of Rabbit That Died of Osteogenic Sarcoma After Inhalation of Beryllium Oxide*

	Milligrams Be per 100 Gm. of Tissue
Lung	213.0
Bone (right femur)	0.0365
Liver	0.622
Kidney	0.135

They have occurred with considerable regularity in rabbits given intravenous injections of poorly soluble compounds of beryllium.

Relative to the tumor which followed the inhalation of beryllium oxide, a question naturally arises as to how inhaled dust induced development of a tumor in a bone of the pelvis. The fact that inhaled compounds of beryllium can be distributed to abdominal organs and can lead to extrathoracic lesions has been demonstrated in human beings suffering from chronic pulmonary berylliosis following inhalation of beryllium oxide or phosphors. Granulomatous lesions have been observed in the liver, the spleen, bone and in extrathoracic lymph nodes of several such patients.[5] Furthermore, chemical analyses have revealed beryllium in such tissues as liver, spleen, kidney, bone and other tissues of patients who died of chronic berylliosis.[6] Analyses of tissues of experimental animals exposed to atmospheres containing poorly soluble compounds of beryllium also show that beryllium is distributed in various quantities through most of the organs of the body.

In patients in whom chronic granulomatosis has developed following inhalation of the poorly soluble compounds of beryllium, microscopic study of the lungs invariably reveals the presence of macrophages in which dust particles can be identi-

4. Cholak, J., and Hubbard, D. M.: Spectrographic Determination of Beryllium in Biological Material and in Air, Anal. Chem. **20**:73-76, 1948; Spectrochemical Determination of Beryllium, ibid. **20**:970-972, 1948.

5. DeNardi and others.[1b] Vorwald.[1c] Dutra.[2a]

6. Dutra, F. R.; Cholak, J., and Hubbard, D. M.: The Value of Beryllium Determinations in the Diagnosis of Berylliosis, Am. J. Clin. Path. **19**:229-234, 1949.

fied. Similarly, in experimental animals exposed to dusts of beryllium oxide, such macrophages are invariably present in the lungs, pulmonary lymphatics and mediastinal lymph nodes. Lymphatics accessible to inhaled beryllium oxide provide a ready route by which it may enter the blood via the thoracic and right lymphatic ducts. It is even possible that some of this fine particulate matter may pass directly through the walls of the alveolar capillaries, from which it would be distributed in the blood to other organs of the body. The fact that in human beings extrathoracic granulomatous lesions have occurred invariably in organs rich in reticuloendothelial cells suggests that the localization of the dust which gains access to the blood stream depends to a large extent on the phagocytic activities of the littoral cells.

The only reported occupational tumors of bone following absorption of chemical substances have appeared in persons who had ingested radioactive materials.[7] During the last 20 years, considerable numbers of persons have been exposed to dusts of poorly soluble compounds of beryllium in various industries throughout the United States. Despite the fact that cases of cancer of this type have not been reported, it is possible that the inhalation of poorly soluble compounds of beryllium

TABLE 2.—*Beryllium in Bone of Persons Who Died of Berylliosis, Compared with That in Bone of Rabbit Which Died of Osteogenic Sarcoma*

	Micrograms Be per 100 Gm. of Bone
Rabbit with osteogenic sarcoma	36.5
Persons with acute berylliosis	0.0 0.3
Persons with chronic berylliosis	0.0* 0.44 1.55 3.0 13.5

* Nonoccupational case.

may eventuate in osteogenic sarcoma in man. Presumably, the incubation period of such tumors would be considerably longer in man than in rabbits, and observations may be required over a period of years before it will be known whether persons who have been exposed to beryllium are prone to have such tumors. Barnes,[8] who has produced osteogenic sarcomas in rabbits by intravenous injection of finely divided metallic beryllium as well as with zinc beryllium silicate, has stated that osteogenic sarcoma from compounds of beryllium "might possibly be another industrial hazard."

There is reason to believe that the possibility of being exposed to significant amounts of beryllium in industry is being well controlled today. The atmospheric concentration of beryllium oxide which led to the development of osteogenic sarcoma in the rabbit may have been somewhat higher than that present in industrial environments at any time in years past. The concentration of beryllium in normal bone of this rabbit was greater than that in the bones of persons who died of berylliosis (table 2). The possibility that osteogenic sarcoma may occur in persons who have

7. Martland, H. S.: Occupational Tumors, Sec. 6, Bones in Occupation and Health, Encyclopedia of Hygiene, Pathology and Social Welfare, supp., Geneva, Switzerland, International Labour Office, 1939.

8. Barnes, J. M.: Experimental Production of Malignant Tumours by Beryllium, Lancet **1**:463, 1950.

been exposed to the inhalation of compounds of beryllium is suggested by the fact that compounds of beryllium are absorbed and may be recovered from bone even after a prolonged illness during which there was no further beryllium exposure.

SUMMARY

A rabbit that had been exposed repeatedly to an atmosphere containing beryllium oxide died of osteogenic sarcoma 17½ months after the last day of exposure. The primary tumor was in the left pubis, and there were widespread visceral metastases.

Similar tumors are readily produced by intravenous injections of poorly soluble compounds of beryllium. Since such tumors have not been observed as a spontaneous condition in our rabbits, it is believed that this sarcoma resulted from the influence of the beryllium compound that was inhaled by this animal.

A comparison of the concentrations of beryllium in normal bone of the rabbit in which the tumor developed and in patients who died of berylliosis forms the basis for concluding that osteogenic sarcomas may eventually develop in some persons who have inhaled poorly soluble compounds of beryllium.

HISTOCHEMICAL DISTRIBUTION OF GLYCOGEN

I. Evaluation of Methods

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DIFFERENT workers [1] have indicated variations in the histochemical distribution of glycogen, depending on the fixation, the staining technic and the tissue employed. Recently, appearances of tissues stained by the several methods outlined below for the demonstration of glycogen were to us reminiscent of the colored chemical tissue precipitates obtained with chlorides and phosphates in 1906 by Macallum and Menten.[2] This parallelism indicates identical loci for these two cellular constituents at least [3] and suggests the presence in tissue of plasmatic units whose composition, polymerization and structure may vary from time to time, depending on changes in local conditions. The considerable amount of glycogen demonstrated in the albuminous casts of nephrotic kidneys implies that the glycogen is combined with the albumin. Furthermore, as salivary digestion does not, with all technics, reduce materially the demonstrable glycogen, the linkage of glycogen and albumin is apparently not easily disrupted. Either alteration of tissues in vivo or disruption of the tissue complexes by hydrolytic agents in vitro is a necessary prelude to positive staining reactions. The degree and the character of such changes modify the final outcome of the histochemical technics for glycogen, thus posing difficulties in the interpretation of the specificity of the various methods devised for localizing this compound in tissue. The introduction of improved technics [4] for the histochemical demonstration of glycogen and the variability we encountered in kidney stained by these technics made a review of the technics in use and evaluation of the conditions of their application desirable.

From the Department of Pathology, University of Pittsburgh, and the Children's Hospital of Pittsburgh.

1. (*a*) Wachstein, M.: Blood **4**:54, 1949. (*b*) Wislocki, G. B., and Dempsey, E. W.: Anat. Rec. **96**:249, 1946. (*c*) Wislocki, G. B.; Rheingold, J. J., and Dempsey, E. W.: Blood **4**:562, 1949. (*d*) Gibb, R. P., and Stowell, R. E.: Ibid **4**:569, 1949.

2. Macallum, A. B., and Menten, M. L.: Proc. Roy Soc., London, s.B. **77**:165, 1906.

3. Using tetrazolium chloride, Zweifach and associates recently described the same type of stain reaction in the proximal convoluted tubules of hypertensive subjects (Zweifach, B. W.; Black, M. M., and Shorr, E.: Proc. Soc. Exper. Biol. & Med. **74**:848, 1950).

4. (*a*) Gomori, G.: Am. J. Clin. Path., Tech. Sect. **10**:177, 1946. (*b*) Hotchkiss, R. D.: Arch. Biochem. **16**:131, 1948.

MATERIALS

Blocks of kidney, liver and spleen removed routinely at autopsy were selected from cases which had been diagnosed clinically and anatomically as glycogenosis (von Gierke's disease), nephrosis, nephritis, pyelonephritis, hydronephrosis, diabetes, leukemia, congenital heart disease and atresia of the esophagus. The ages of the patients at death varied from 5 days to 14 years. A few biopsy specimens of sternal bone marrow were also studied. Descriptions of the findings are presented in subsequent papers.[5]

FIXATIVES

The fixatives used included the following:

Formaldehyde solution U. S. P. diluted 1:10.

Ninety-five per cent alcohol.

Zenker's solution: Potassium dichromate, 0.5 Gm.; mercury bichloride, 0.6 Gm.; glacial acetic acid, 5 ml.; water, 95 ml.

Ninety-five per cent alcohol, 80 ml.; formaldehyde solution U. S. P., 5 ml.; glacial acetic acid, 5 ml.; water, 5 ml.

Bouin's fluid: Saturated picric acid, 5 ml.; formaldehyde solution U. S. P., 5 ml.; acetic acid, 10 ml.; water, 80 ml.

Formaldehyde solution U. S. P., 5 ml.; glacial acetic acid, 5 ml.; water saturated with picric acid, 90 ml.

Water saturated with picric acid, 5 ml.; formaldehyde solution U. S. P., 5 ml.; acetic acid, 10 ml.; 95 per cent alcohol, 80 ml.

Acetone.

From tissues removed 45 minutes to six hours after death, frozen and paraffin sections were prepared.

The majority of tests described for the localization of glycogen react with only a fraction of the molecule. The kind of fixative used may modify this fraction chemically; e. g., formaldehyde solution increases reduction.[4a] After having examined material prepared by the Altmann-Gersh freezing-drying method, Gersh [6] suggested that glycogen is distributed throughout the cell; later work [7] suggests that it exists as a colloid which is precipitated in granular form when fixatives are used. Kulonen [8] supplied evidence that chromic acid precipitates glycogen while Lison [9a] suggested that there may be adsorption on a protein substrate. Therefore, the kind of fixative, e. g., alcohol or formaldehyde solution, may modify the complex produced, so that after fixation the stain reacts not with glycogen per se but with one of its degradation products.

Formerly it was believed that glycogen could be demonstrated only in tissues fixed in alcohol. We, however, corroborate the finding of Vallence-Owen [10] that glycogen is demonstrable in tissues fixed in formaldehyde solution and after vigorous washing in water. Meyer [11] reported that the greater the degree of polymerization

5. Menten, M. L., and Carpenter, A. M.: A. M. A. Arch. Path., this issue, pp. 486 and 494.

6. Gersh, I.: Anat. Rec. **53**:309, 1932.

7. Lazarow, A.: Science **95**:49, 1942.

8. Kulonen, E.: Acta path. et microbiol. scandinav. **27**:461, 1950.

9. Lison, L.: Histochimie animale: Méthodes et problèmes, Paris, Gauthier-Villars, 1936, (*a*) p. 228; (*b*) p. 227; (*c*) p. 231.

10. Vallence-Owen, J.: J. Path. & Bact. **60**:325, 1948.

11. Meyer, K. H.: Advances Enzymol. **3**:109, 1943.

of the glycogen, the less water-soluble it becomes. It is possible, too, that there are variations in the solubility of glycogen in different tissues as Ehrlich [12] demonstrated in bone marrow.

We were impressed by the results obtained by adding water saturated with picric acid to the fixative as suggested by Lison [9b] for both the iodine and the Bauer-Feulgen technics. This adjunct produced, as well, especially sharp colors with the Hotchkiss technic, but there was no stain after Gomori's silver nitrate-methenamine technic.

Time of fixation after death and time of staining subsequent to fixation did not appear to be limiting factors. Blocks of tissue representing von Gierke's disease which were fixed and stained for glycogen in 1945 gave comparable quantitative reactions four years later.

On the whole, in a pathology laboratory, formaldehyde solution U. S. P. (formalin®) serves well as a fixing fluid for tissue glycogen because no special attention or foresight is necessary; material can be utilized at any time even though fixation was instituted years before, and it is possible to use two excellent staining technics, viz., the Hotchkiss technic and Gomori's silver nitrate-methenamine, which serve as controls for each other.

STAINS

Staining technics employed to demonstrate glycogen histochemically were the iodine,[13] Best's carmine,[14a] the Bauer-Feulgen,[14b] the periodic acid-Schiff technics of McManus,[15] the Wislocki,[1c] the Hotchkiss,[4b] and the silver technics of Mitchell and Wislocki [16] and Gomori.[4a] Parallel studies for demonstrating alkaline phosphatase were made on acetone or alcohol-fixed material by the methods of Menten and associates,[17] Gomori [18] and Nachlas and Seligman.[19]

The iodine and Best's carmine stains are empiric reactions. Later-published methods utilize, for the development of color, an aldehyde group set free from the carbohydrate by acid treatment. The dye employed, generally the fuchsin-sulfurous acid reagent, is reduced. The reacting aldehyde may be derived from sugars other than glucose of glycogen, as has been shown by Morrison [20] with galactose in Gaucher's disease; hydroxyamino acids also react.[21] The Bauer-Feulgen technic freeing aldehydes by chromic acid pretreatment yields a diffuse stain regardless of the fixative, and there is a nonspecific tinting of the background. The Hotchkiss modification in which periodic acid is substituted as the hydrolytic agent not only shows especially pronounced reactions after alcohol fixation but also gives as excellent coloration after formaldehyde fixation. Reacting substances which

12. Ehrlich, P.: Ztschr. klin. Med. **6**:33, 1883.
13. Bensley, C. M.: Stain Technol. **14**:47, 1939.
14. Glick, D.: Techniques of Histo- and Cytochemistry, New York, Interscience Publishers, Inc., 1949, (*a*) p. 49; (*b*) p. 48; (*c*) p. 17; (*d*) p. 57; (*e*) p. 58; (*f*) p. 59.
15. McManus, J. F. A.: Nature, London **158**:202, 1946.
16. Mitchell, A. J., and Wislocki, G. B.: Anat. Rec. **90**:261, 1944.
17. Menten, M. L.; Junge, J., and Green, M. H.: J. Biol. Chem. **153**:471, 1944.
18. Gomori, G.: Am. J. Clin. Path. **16**:347, 1946.
19. Nachlas, M. M., and Seligman, A. M.: J. Nat. Cancer Inst. **9**:415, 1949.
20. Morrison, R. W., and Hack, M. H.: Am. J. Path. **25**:597, 1949.
21. Nicolet, B. H., and Shinn, L. A.: J. Am. Chem. Soc. **61**:1615, 1939.

underlie the development of color are ably discussed by Hotchkiss,[4b] but Jeanloz [22] questioned the validity of the assumption that the requirements for positive results with the Hotchkiss tests are two adjacent free hydroxyl groups.

Gomori recommended chromic acid pretreatment to form the polyaldehyde and subsequent reduction of silver nitrate. This technic, applicable after either alcohol or formaldehyde fixation, is the most sensitive in relation to the amount of glycogen present, showing differences of less than 0.5 Gm. per 100 Gm. of tissue.[23] Its variability seemingly bears a relationship to the metabolic state of the cytoplasm. There is a possibility that glucose phosphate intermediates, as well as insoluble inorganic phosphates which might be present in tissue, react to form the colored product.

Our results are in agreement with the suggestion of Lison [9c] that concentration and time of exposure are factors in the application of chromic acid. It is our belief that test slides are needed for each case studied. We advise a series of four slides, which should be removed from the chromic acid, one at a time after 5, 15, 30 and 60 minutes. The type of vessel used for incubation of tissues affixed to slides in silver nitrate-methenamine solution should provide adequate space between and above the slides, so as to allow free and constant exchange of air throughout the fluid; otherwise the incubation period is prolonged or the reaction does not occur at all.

With either the Hotchkiss or the Gomori technic, the solutions freshly made according to the directions of the respective authors gave the best results.

It may be pointed out finally that two types of reaction occur in pathologic tissues treated with the Hotchkiss stain. In renal tissues showing glycogenosis (von Gierke's disease) and in diabetes a film of stain appeared to be superimposed on the surface as globules, comparable to the film formation seen with Best's carmine stain. In tissues from kidneys the condition of which was diagnosed as nephrosis the stain was located as granules of varying size, density and color intensity. This difference was not seen after silver nitrate-methenamine staining.

Our failure to obtain histochemical evidence of the presence of alkaline phosphatase in the tissue examined was disappointing in view of the polemic on the relationship which this enzyme bears to glycogen.

CONTROL PROCEDURES

Control procedures as enumerated below were instituted in order to assess the validity of the histochemical reactions. Many of the results were difficult to evaluate.

(*a*) To rule out preformed aldehydes or interfering substances, tissues were stained without being exposed to periodic or chromic acid. Slides stained without being pretreated with hydrolyzing agents should have been negative; after formaldehyde or alcohol fixation the Hotchkiss technic without periodic acid revealed no color, but the Gomori technic with deletion of chromic acid showed blood cells [24]

22. Jeanloz, R.: Science **111**:289, 1950.

23. Bondy, P. K.; Sheldon, W. H., and Evans, L. D.: J. Clin. Invest. **28**:1216, 1949.

24. It should be noted that the blood cells here alluded to, as well as those described later in pathologic renal conditions, were chiefly erythrocytes. In most cases it was not possible to differentiate the various leukocytes.

of a very pale fuchsia hue. In the latter case the formation of color was undoubtedly due to preformed aldehydes or interfering substances. Furthermore, blood cells in alcohol-fixed tissues of patients dying of von Gierke's disease, when subjected to an identical Gomori technic (i. e., minus chromic acid), also stained. We believe this confirms the impression that the fixative was not responsible for release of the reacting aldehydes but rather that these were preformed.

(*b*) Slides left 24 hours in 95 per cent alcohol to remove alcohol-soluble plasmal reacted similarly to those which were not subjected to the alcohol bath. Conversion of plasmalogen to plasmal is seemingly not a factor in the interpretation of results.

(*c*) To determine the effectiveness of agents used in pretreatment, periodic was substituted for chromic acid in the Gomori technic and chromic for periodic acid in the Hotchkiss method. No color appeared when chromic acid was substituted for periodic acid in the Hotchkiss technic. However, when periodic acid was substituted for chromic acid in the Gomori technic, the basement membranes became intensely black. Apparently periodic acid is the more active hydrolyzing agent.

(*d*) The variable action of different enzymes in the removal of glycogen was likewise difficult to interpret. Enzymic predigestion to remove glycogen was undertaken with repeated changes of saliva [13] for 45 minutes at 37 C. or with amylopsin [1c] in phosphate buffer at pH 6.8 for 30 minutes at 37 C. Saliva was not uniformly potent in preventing the development of stain by either the Hotchkiss or the Gomori technic, because stain was observed occasionally in the beaded line, often in the basement membrane and in the detritus lying in the lumens of the tubules. Differences were also noted in a series in which saliva was used in parallel with amylopsin. Neither saliva treatment nor amylopsin treatment prevented color reaction in blood cells in tissues subsequently treated with the silver nitrate-methenamine technic. Saliva was less active than amylopsin in preventing the color formation with the Hotchkiss technic. Thus in alcohol-fixed material amylopsin rendered negative all stainable components, in contrast to the inability of saliva to produce similar negative changes. These results corroborate findings of others: Wislocki, Rheingold and Dempsey [1c] found, with the periodic acid-Schiff method, that the basement membrane and reticulum fibers stained following salivary digestion but did not stain after maltase treatment. Wachstein,[1a] quoting Neukirch, stated that leukocytes and platelets stained with iodine and Best's carmine after salivary digestion. With the Bauer-Feulgen technic, Wislocki and associates [1c] also found that these blood cells did not stain following pretreatment with saliva. Our results agree with the finding of Wachstein [1a] that air-dried films of bone marrow are less easily attacked by saliva than films which have been alcohol-fixed and that stains following salivary digestion on films of marrow from the same patient are variable.

(*e*) A number of approaches were employed to demonstrate inorganic elements and radicals. These included removing insoluble calcium with citrate buffer [4a] at pH 4. Cretin's [14c] histochemical technic was tried for calcium and Macallum's [2] method for phosphate and chloride. After Gomori's technic for the removal of preformed calcium had been applied, all staining with silver nitrate-methenamine failed. Untreated duplicate sections when subjected to Cretin's technic for calcium showed only the faintest diffuse nonspecific coloration. The question then arose whether preformed calcium was the moiety removed in the first case, or what alteration may have taken place in the tissues.

Histochemical tests for both phosphate and chloride were positive in loci the same as those described for glycogen. Macallum's [25] report, that eight precipitations with ammonium sulfate are required to free chloride when it is bound to a colloid, was borne out by our positive findings when the Hotchkiss reagent was used in vitro on lactalbumin after prolonged dialysis.

(*f*) To demonstrate possible glycogen-protein linkage, the methods of Serra [14d] and of Thomas [14e] for arginine and of Millon [14f] for tyrosine were employed. Analogous inconclusive findings were obtained for these associated substances. The three methods of localizing protein components gave similarly very faint nonspecific coloration. It is suggested that the hydrolytic agents in the technics employed were not sufficiently active either to disrupt the tissue complexes or to free the reacting protein constituent parts. A further possibility is a redistribution after this liberation. The fact that glycogen stains are always markedly positive in renal casts, a locus for protein, lends support to a combination of glycogen and protein. Such an association is especially pronounced in diseases of the kidney in which albumin is the moiety abnormally excreted. The inadequacy of salivary digestion in preventing staining strongly indicates that the linkage cannot be broken easily.

(*g*) Test tube reactions were undertaken to determine the specificity of Gomori's silver nitrate-methenamine technic with solutions of Lactamin (Wyeth's lactalbumin hydrolysate) before and after dialysis against distilled water. For the Hotchkiss stain glucose, uric acid and alcohol were similarly tested. Acetates, chlorides, nitrates and orthophosphates of ammonium, calcium, potassium and sodium were tested in like manner with both stains.

All the above "specificity" tests for glycogen with the silver nitrate-methenamine reagents were positive. With the exception of the phosphates, the inorganic chemicals, because of their solubility in water, should have posed no problem in the histochemistry of tissue. Sodium, calcium and potassium orthophosphates were only slightly soluble in water. The chlorides were soluble; in spite of this fact they were demonstrated histochemically, possibly because they were intimately and strongly bound with other tissue components. A comparable explanation probably suffices for the persistent slight reaction of the amino acids or polypeptides of lactalbumin after prolonged dialysis of one week. With the Hotchkiss technic, lactalbumin, glucose and uric acid gave negative reactions after 18 hours' dialysis.

It is interesting that alcohol per se reacted with the Schiff reagent. But, inasmuch as slides which had not been pretreated with periodic acid were negative, the alcohol itself did not account for the positive results seen in tissues.

CONCLUSIONS

It is difficult to ascribe specificity to the staining technics used for the histochemical localization of glycogen because of the variable results obtained. This variability may be ascribed in part to differences in the structure and composition of tissue complexes which contain glycogen and which may be modified by local physiologic or pathologic conditions. Another factor underlying variability of result is a technical one, namely, the type of hydrolytic agent incorporated in the method designed to release moieties, which act directly with the staining reagent, from the tissue complexes. Other modifying factors are discussed.

25. Macallum, A. B.: Proc. Roy. Soc., London, s.B. **76**:217, 1905.

HISTOCHEMICAL DISTRIBUTION OF GLYCOGEN

II. In Kidneys of Patients with Different Pathologic Conditions, Especially Those with Lipoid Nephrosis

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RESORPTION, by the subtending tubules, of a considerable part of the glucose which is normally excreted through the glomerular filter is an important feature of the conservation of the carbohydrate in the body. The actual changes that occur as substances pass through the tubular epithelium are not definitely known, but the complex process of glucose transport apparently takes place through a series of phosphorylations. We thought it possible that a comparative study of pathologic renal conditions using improved histochemical technics for the localization of glycogen or its precursors might shed light on the reabsorptive transport of glucose. The results of such a study made on kidney tissues and on urine are outlined in the following pages.

MATERIAL AND METHODS

Kidney tissues removed routinely at autopsies were selected from cases in which the anatomic diagnoses were nephritis, pyelonephritis, nephrosis, hydronephrosis, diabetes and glycogenosis (von Gierke's disease). Control procedures were performed on sections from kidneys not primarily involved in a disease process. All tissues were fixed in solution of formaldehyde U. S. P. diluted 1 : 10, and those from cases of von Gierke's disease in 95 per cent alcohol as well. Paraffin sections were cut at 3 to 5 microns; when desirable, frozen sections were made.

The technics used for the histochemical demonstration of glycogen were the iodine,[1] Best's carmine,[2a] the Bauer-Feulgen,[2b] the Hotchkiss' modification[3] of the periodic acid-fuchsin-sulfurous acid method, and Gomori's[4] alkalinized silver nitrate-methenamine technic. In addition, studies were made to determine the specificity of material demonstrated as glycogen. These tests included removing preformed calcium from tissue with citrate buffers at pH 3.5 to 5.7[4] and predigesting glycogen in tissue both with salivary amylase[1] at 37 C. for 45 minutes and with amylopsin in phosphate buffer[5] at pH 6.8. Phosphates and chlorides were demonstrated, as well.[6] A critique of these methods is presented in the preceding publication.[7]

From the Department of Pathology, University of Pittsburgh, and the Children's Hospital of Pittsburgh.

1. Bensley, C. M.: Stain Technol. **14**:47, 1939.

2. Glick, D.: Techniques of Histo- and Cytochemistry, New York, Interscience Publishers, Inc., 1949, (a) p. 49; (b) p. 48.

3. Hotchkiss, R. D.: Arch. Biochem. **16**:131, 1948.

4. Gomori, G.: Am. J. Clin. Path., Tech. Sect. **10**:177, 1946.

5. Wislocki, G. B.; Rheingold, J. J., and Dempsey, E. W.: Blood **4**:562, 1949.

6. Macallum, A. B., and Menten, M. L.: Proc. Roy. Soc., London, s.B **77**:165, 1905-1906.

7. Carpenter, A. M.; Polonsky, B., and Menten, M. L.: A. M. A. Arch. Path., this issue, p. 480.

Comparable experiments were carried out on urine from a patient (N. W.) with nephrosis. Casts, precipitated protein and fractions removed following electrophoretic separation were tested for glycogen by the Hotchkiss [3] and Gomori [4] technics. Parallel studies were done on albumin-free urine from a patient (J. M.) who three years previously had recovered from nephrosis following exposure to measles.[8]

HISTOCHEMICAL OBSERVATIONS

Positive color development was obtained constantly with the various technics, both in normal control and in pathologic tissues, in the basement membrane throughout the nephron as well as in the interna elastica, the elastic fibrils of the media and the reticulum fibers of the tunica adventitia of the arteries. In normal tissues no glycogen was demonstrated elsewhere in the kidney. In pathologic tissues there was, besides, a considerable deposit in the tubules, in the cells of which the amount and distribution varied, depending on the condition present. The most intense reaction was seen in von Gierke's disease, but many nephrotic kidneys showed an almost equally intense color. In kidneys from patients who had died of diabetes or nephritis, only a moderate tinting was obtained. The distribution of the glycogen in the different abnormal renal states examined is described below in some detail.

Parenchymatous Degeneration of Kidneys.—The cytoplasmic granules of the epithelium lining the convoluted tubules, after the application of Gomori's silver nitrate-methenamine technic, took on a violet-gray color, the intensity of which deepened with advancing grade of degeneration. Occasionally the mitochondria were outlined as parallel lines of granules. Frequently, in advanced cloudy swelling, the beaded line and the ciliated region luminal to it were also colored. After tissues had been treated with saliva, the color was retained only in the basement membrane and the beaded line, although it appeared more diffuse.

Glycogenosis.—In von Gierke's disease, as might be expected, the tubular epithelium showed a marked concentration of glycogen, varying in amount and color with the technic employed. With all technics the proximal and distal convoluted tubules and the ascending limb of the loop of Henle contained the greatest quantity of stainable material. The reacting globules differed widely in size and distribution but were least in amount in the large vacuolated epithelial cells. The collecting tubules showed a faint color resident in finely dispersed granules. The small spherical granules of the beaded line stood out prominently. The maximal color, of a violet hue, was obtained with the Hotchkiss technic. Intensity of stain was so great with Best's carmine that the large coalesced globules appeared almost black. With Gomori's silver nitrate-methenamine, after alcohol fixation, the reaction was less intense, and the violet-black coloring of the tubular epithelium was in contrast to the reddish tone in the basement membrane of the glomerulus and the walls of the vessels. The glomeruli generally showed an irregular distribution of color apparently present in the basement membrane, but color formation occasionally appeared diffuse, possibly owing to a slight redistribution. Except for an occasional outlining of a nucleus, the epithelium lining the capsular space was negative. Blood cells [9] stained red with silver nitrate-methenamine but were variable with other technics. Pretreating the tissue with saliva eliminated color from all sites except

8. Meizlik, E. H., and Carpenter, A. M.: Am. J. Dis. Child. **76**:83 (July) 1948.

9. The term "blood cells" is used collectively as explained in paper I.

the basement membrane and the vascular walls. After removal of preformed calcium [4] no color remained anywhere in renal tissue.

Nephrosis.—In renal tissue from patients who had died with nephrosis, best results were obtained with silver nitrate-methenamine. Under low power the positive pattern was distributed irregularly throughout the cortex, chiefly in the convoluted tubules and the ascending limb of the loop of Henle. In those nephrons which were undergoing degenerative changes, the granules were large and intensely colored (fig. 1). In the normal-appearing tubules the color was minimal through-

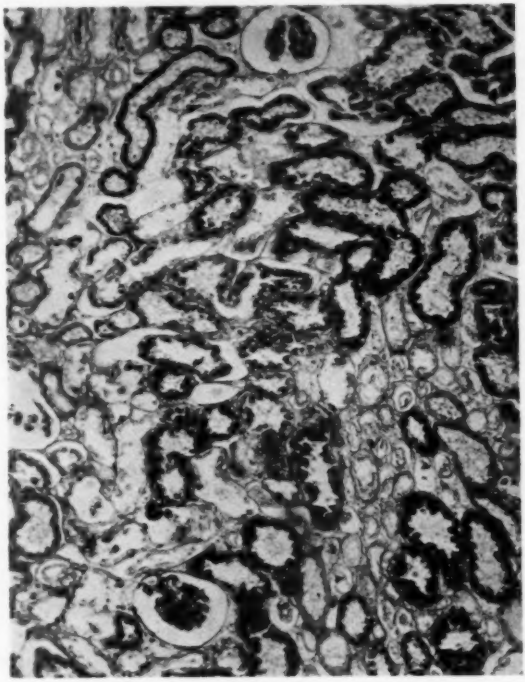

Fig. 1.—Photomicrograph (× 100) to illustrate the typical reaction of the silver nitrate-methenamine stain in nephrotic kidney.

out (fig. 2*A*). The reaction first developed in the beaded line and was seen occasionally in the outer border of the cilia (fig. 2*B*). The first components of the cytoplasm to be involved were the mitochondria (fig. 2*C*), but gradually the reactive substance became increased in volume and aggregated (fig. 2*D*). The accumulation of varying-sized granules in the cytoplasm, many of them large, became so great that the beaded line was not discernible. A row of fine granules, interrupted here and there, outlined the luminal border of the cilia. The peripheral half of the tubular epithelium was practically colorless, as were also the nuclei. Gradually, as the granules in the inner half of the tubular cells became larger and more irregular in distribution, a diffuse pale brown tinting developed in the outer half of

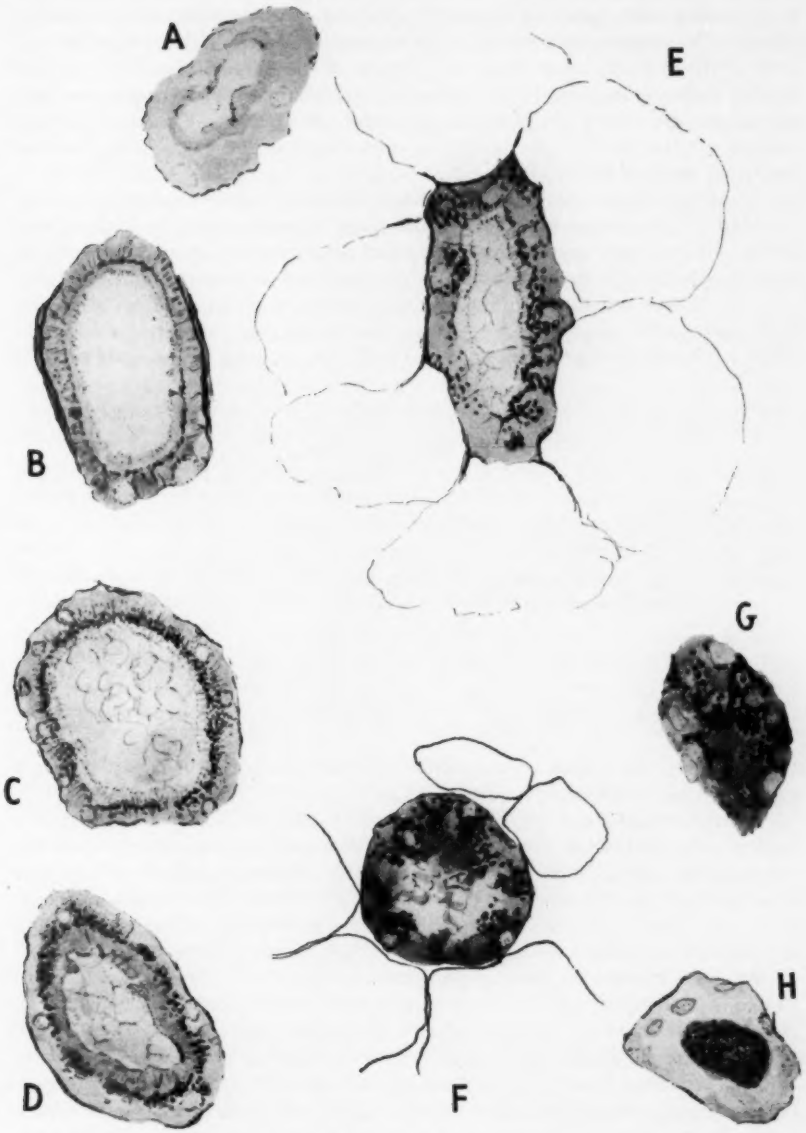

Fig. 2.—Drawings (× 500) showing progressively increasing quantities of glycogen in the proximal convoluted tubules.

their cytoplasm (fig. 2 *E*). Conversion of the homogeneous nonstaining cytoplasm to a granular state continued (fig. 2 *F*) until the structure of the tubules became altered. The granules then had an irregular distribution within the tubular outline; finally, with diffusion of contents and rupture of the cell wall (fig. 2 *G*), all the reacting material appeared in the lumen of the tubule, where the granules, staining an intense black, were embedded in a grayish brown matrix. The area originally occupied by the cytoplasm of the tubular epithelium was devoid of color except for the faintly outlined nuclei (fig. 2 *H*).

The glomeruli varied in size and vascularity, and the basement membrane stained intensely. The basement membrane of the dilated capsular space also stained positively. The walls of vessels showed the usual positive stain. Blood protein which had passed through the glomerular filter appeared as intensely brown-staining granular casts in the lumens of the collecting tubules, while the surrounding lining cells were devoid of color. After salivary predigestion and after removal of preformed calcium, all stain was lost. Blood cells were positive for glycogen with all

Results of in Vitro Experiments Using Histochemical Methods to Demonstrate Glycogen

	Nephrotic Urine from N. W.		Albumin-Free Urine from J. M.[9]	Lactalbumin Hydrolysate
	Casts	Precipitated Protein		
Hochkiss technic [3]				
Before dialysis	++	+++	++	++
After dialysis	..	+	−	−
After salivary digestion	+	+	..	..
Gomori technic [4]				
Before dialysis	+	+	..	++++
After dialysis	..	+	..	+
After salivary digestion	−	−	..	..
Iodine stain [1]	−	+	−	−
Phosphate stain [6]				
Before dialysis	+	+	..	+
After dialysis	..	−	..	−
Chloride stain [6]				
Before dialysis	+	+	..	−
After dialysis	..	−	..	−

technics except silver nitrate-methenamine after alcohol fixation or alcoholic picrate subsequent to formaldehyde fixation.

In the investigation of urine from nephrotic patients, the casts and precipitated protein were positive in vitro (table) with all technics for glycogen except the iodine, which was not sufficiently sensitive for the amounts contained in the urine. Subsequent to salivary treatment no color was formed with silver nitrate-methenamine, and with the Hotchkiss technic the color was decidedly reduced. Following dialysis against distilled water, precipitated urinary protein stained for glycogen [10] but was colorless after technics for phosphates and chlorides.[6] Glycogen was demonstrable in all fractions of the urinary protein after electrophoretic separation. The electrophoretic fractions, namely, albumin and alpha, beta and gamma globulins, reacted before and after dialysis against water. Albumin-free urine from a patient who had recovered from nephrosis [8] was also colored with all technics for glycogen, but the reaction disappeared after dialysis. This positive result in the undialyzed urine may have been due to amino acids [11] present, inasmuch as a lactalbumin hydrolysate was also positive before dialysis but negative after prolonged dialysis.

10. Hotchkiss,[3] Gomori.[4]

11. Nicolet, B. H., and Shinn, L. A.: J. Am. Chem. Soc. **61**:1615, 1939.

Diabetes.—Positive staining was obtained with silver nitrate-methenamine in the basement membrane, the arteries and the collecting tubules. The epithelium of the latter was shrunken and separated from the basement membrane, probably because of fixation. The nuclei appeared as colorless spots. Cells lining the convoluted tubules and the ascending limb of the loop of Henle, including the enlarged Armanni-Ehrlich type, were also practically colorless. Amylopsin prevented all color formation except in blood cells.

With the Hotchkiss technic, in addition to the positive reaction in the basement membranes and the walls of arteries, colored globules were seen in tubular cells which were undergoing granular degeneration. In a considerable number of tubules, desquamated cells appeared as detritus in which a collection of deep purple globules of varying size was prominent. The intact, normal-appearing cells displayed little or no reaction. Amylopsin completely prevented all stain.

Nephritis.—In the acute stage of this disease the only positive formation noted was in the basement membrane of tubules and in the arterial walls. During the early part of the third stage, in addition to the above findings, a red-brown color appeared in tubular casts and in fibrils resembling elastic tissue throughout the enlarged glomeruli. The blood cells were colored a dark red-brown.

Pyelonephritis.—Localization of glycogen persisted in the tubular basement membrane and in arterial walls where these structures had not been destroyed by the ascending infection. In the areas of remaining intact nephrons no color was present. The reaction of these tissues involved in the infection waned and disappeared as the degenerative process gradually advanced, with loss of kidney nephrons and their replacement with fibrosis. Blood cells stained deeply.

Hydronephrosis.—In this condition it is interesting that the finely granular material in the dilated tubules showed no tinting. The lining epithelium of the tubules was also negative, but the basement membrane, especially that of the glomeruli, not only appeared thickened but also revealed an enhanced stain.

COMMENT

The question of specificity of staining technics for glycogen has been discussed in a previous paper.[7] In this paper all positive-staining reaction will be considered as indicating glycogen or a closely allied polysaccharide. Our studies corroborate those of Wachstein,[12] who found no regular or constant distribution of glycogen in the kidney of von Gierke's disease. We have extended this finding, using refined technics, to include other pathologic renal conditions.

The histochemical demonstration of renal glycogen may vary in relation to a number of conditions, namely, (1) the morphological development of the organ,[13] (2) the kind of material present [14] and the state in which it exists, and (3) the pathologic condition of the organ.

Hinde,[13] working on laboratory animals, reported that in the first three weeks of life glycogen was limited to the collecting tubules—that part of the kidney derived from the metanephric duct. Morrison and Hack [14] observed that in Gaucher's disease the positively reacting kerasin contained in the large foam cells could not be

12. Wachstein, M.: Am. J. M. Sc. **214**:401, 1947.
13. Hinde, I. T.: J. Path. & Bact. **61**:451, 1949.
14. Morrison, R. W., and Hack, M. H.: Am. J. Path. **25**:597, 1949.

rendered negative by various solvents. In contrast, solvents readily deleted the positive reaction obtaining in the kerasin phagocytosed by similar monocytes after experimental injection of this compound. The difference may have been due to variation in the degree of polymerization of the kerasin, to combination of kerasin and some other substance or to variation in chemical structure.

In our studies, the relation of pathologic change to positive color development was noted in different diseases. Salivary predigestion prevented all color formation, including that in elastic tissue and basement membrane, in diabetes and nephrosis, in contrast to the refractoriness to enzymic digestion seen in von Gierke's disease, parenchymatous degeneration and nephritis. In the first two diseases the chemical structure of the basement membrane may have become more labile and modifiable. The gradual loss of staining of basement membrane and elastic tissue in the nephrons involved in the ascending infection in pyelonephritis is a further indication of plasticity of this structure. It is interesting that in hydronephrosis the basement membrane showed increased intensity of stain. These findings are in agreement with Gersh's [15] conception of the chemical lability of the basement membrane. According to McManus,[16] the substance detectable in this structure is probably of the nature of chitin. We believe that the cytoplasm of the tubular epithelium possesses an even greater inherent capacity than the basement membrane for alteration of chemical state as indicated by differences in demonstrable glycogen exhibited in various diseases, e. g., in nephrosis as compared with diabetes. It may be noted that the Armanni-Ehrlich cells occurring in human diabetes, commonly believed [17] to contain a maximal amount of glycogen, were completely negative with all technics used in this study.

Parenchymatous degeneration, diabetes, lipoid nephrosis and glycogenosis (von Gierke's disease) form a progressive series with increasing amounts of renal glycogen which can be demonstrated histochemically. In the first condition the glycogen increased with the degree of degeneration. In diabetes, also the glycogen in the tubular epithelium seemed to be definitely related to a retrograde process. In lipoid nephrosis this relationship was even more impressive. In von Gierke's disease the lumen of the nephron was loaded with an excess of glycogen. In these diseases, and especially in lipoid nephrosis, the glycogen is obviously associated with protein or other substances, a condition which has already been suggested by Willstaetter and Rhodewald.[18] We have obtained proof for such a glycogen-protein combination by the electrophoretic experiment on urinary albumin excreted in nephrosis. Blix, Tiselius and Svensson,[19] likewise, previously have shown by electrophoresis that more than half of the total lipids and carbohydrates of the serum is bound to the albumin and the gamma globulin. These are the proteins which are excreted in excess through the glomeruli and tubules in lipoid nephrosis. Blix [20] pertinently noted that the substances carried with different serum proteins influence

15. Gersh, I.: Federation Proc. **7**:270, 1948.

16. McManus, J. F. A.: Federation Proc. **7**:275, 1948.

17. Oliver, J., in Harvey Lectures, 1944-1945, Lancaster, Pa., Science Press Printing Company, 1945, vol. 40, p. 102; Am. J. Med. **9**:88, 1950. Boyd, W. A.: A Textbook of Pathology: An Introduction to Medicine, ed. 5, Philadelphia, Lea & Febiger, 1947, p. 589.

18. Willstaetter, R., and Rhodewald, M., cited by Wachstein, M.: Blood **4**:54, 1949.

19. Blix, G.; Tiselius, A., and Svensson, H.: J. Biol. Chem. **137**:485, 1941.

20. Blix, G.: J. Biol. Chem. **137**:495, 1941.

the transport conditions of each other and that these conveyed substances are in a labile equilibrium which is easily altered. Such changes, he further remarked, may have far reaching consequences in pathologic conditions. The abnormal filtrate in the tubules undoubtedly occasions adverse conditions in the adjoining epithelium producing a variation in colloidal state and altered metabolism with a resultant delay in epithelial transport and stasis of glycogen, as well as of associated substances. An analogous explanation might be advanced for the increase of glycogen reported by Hinde [13] in the terminal portion of the collecting tubules in the vicinity of the papillae. For a short time after birth this part of the tubule is exposed to increased excretory substances, e. g., uric acid, which causes a slight irritation in the lining tubular epithelium and surrounding tissue and manifests itself as uric acid infarct.

The mechanism underlying formation of a state in which visualization of the increased glycogen in the pathologic tissues is possible may be that of a liberation of masked material in which polymerization is high. It may, moreover, arise from increased absorption associated with delayed metabolism and transmission of materials by pathologically altered epithelial cytoplasm. Regardless of the nature of the carbohydrate component or of the site at which it reacts with the various histochemical reagents, the increase in color occurs only in that renal cytoplasm in which retrograde changes have taken place.

SUMMARY

The histochemical distribution of glycogen in the kidneys of patients who died with diabetes, von Gierke's disease, pyelonephritis, nephritis and lipoid nephrosis increased with advance of degenerative change. In lipoid nephrosis the glycogen was in intimate association with the excreted albumin and globulin.

The electrophoretic analyses were made in Dr. Marie A. Fischer's laboratory with the assistance of Dr. Dushan Pavlovich. Dr. M. D. Silverberg permitted us to study his patient, N. W., at the Montefiore Hospital in Pittsburgh.

HISTOCHEMICAL DISTRIBUTION OF GLYCOGEN

III. In Kidneys of Leukemic Patients

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IN OUR previous study [1] on the distribution of glycogen in kidneys from patients who had died of clinically and anatomically diagnosed renal disease, organs from three patients who had died of leukemia were studied as controls. The kidneys from the leukemic patients showed a marked but irregularly distributed increase of the glycogen content of the convoluted tubules and the ascending limb of the loop of Henle. A detailed study was then made of the abdominal organs from 11 children who had succumbed to leukemia, all of whom had been treated. The findings in the renal tissue in these cases are described herein.

In the literature we have found no reference to studies on the distribution of glycogen in the kidneys of patients with leukemia, either treated or untreated.

MATERIAL AND METHODS

In 4 of the 11 cases studied the leukemia was myelogenous in origin; in the remainder it was lymphatic. The age of the patients ranged from 1.5 months to 14 years. The acuteness of the leukemias varied. Nine of the patients received aminopterin, 1 received urethan and another, radioactive phosphorus (P32). The total dose of aminopterin per patient ranged from 2 to 83 mg., and the duration of treatment varied between three and 468 days. Each of the 11 patients had received blood transfusions (table). The technics employed for the demonstration of glycogen were those already described in a previous paper.[2]

HISTOCHEMICAL OBSERVATIONS

The outstanding characteristic throughout the preparations was a frank variation in the quantity of glycogen found in individual cases. The color reactions in the kidney tissue ranged from an absence of any stain to an intense black, and these differences occurred not only in the tubular cytoplasm but also in the blood cells,[3] the basement membrane and the elastic tissue of the vessels. The intensity of color had no apparent relation to the type of therapy except that pronounced staining was noted in the basement membrane where salutary response to aminopterin was prompt.

From the Department of Pathology, University of Pittsburgh, and the Children's Hospital of Pittsburgh.

1. Menten, M. L., and Carpenter, A. M.: A. M. A. Arch. Path., this issue, p. 486.

2. Carpenter, A. M.; Polonsky, B., and Menten, M. L.: A. M. A. Arch. Path., this issue, p. 480.

3. The term "blood cells" is used collectively, as explained in the first paper of this series.

The maximal reaction in the nephron occurred in the proximal convoluted tubules and in the ascending limb of the loop of Henle. Regardless of the technic employed, renal tissues from the patients with myelogenous leukemia showed a greater tendency to react than did those from patients with lymphatic leukemia, where, in any section examined, the stain was patchy. Furthermore, the reaction in leukemic kidneys never attained the intensity of color found in those showing nephrosis, nor was the distribution so widespread. The earliest and most constant change occurred in the beaded line, which in most instances showed an incomplete row of granules.[4] Rarely was the cytoplasmic staining so extensive that the beaded line was obscured. The cellular deposition of color never reached the stage where there was aggregation of granules in the lumen, as in nephrotic kidney.[5] Casts and desquamated epithelium, when present, showed a definite color.

One of the most interesting features found in this study was the visualization of the blood cells in the lumens of the vessels, particularly with the silver nitrate-methenamine technic. The deepest color was found in the more immature cell types of leukemia, including both lymphoblastic and myeloblastic leukemias. The red blood cells varied in color from the most intense black stain obtained anywhere in leukemic tissue, through a dark red, a brownish red, to a complete absence of color in about 10 per cent of the cells. The specific identification of white blood cells was difficult, but in general the ensuing observations agree with those of Gibb and Stowell.[6] The nucleated cells were variable in their staining. In many cases the nuclei remained uncolored; in others the nucleus was black while the cytoplasm was gray-black. Granules were difficult to appraise; they appeared larger in size and coarser than neutrophilic granules. With the Hotchkiss technic, the various blood cells displayed shades of red gradually diminishing to an absence of color.

The basement membrane stained variably with both the silver nitrate-methenamine and the Hotchkiss technic. It is interesting that after the fourth month of duration of the disease the reaction in the basement membrane appeared more variable and finally was negative except in the tissue of patient L. S., where, after remission following aminopterin therapy, the basement membrane outlining the tubules stained deeply.

COMMENT

The reaction for glycogen described in the kidney in leukemia differed from that in nephrosis only in the reduced intensity of staining and in the greater irregularity of distribution. From section to section in slides from the same kidney there was variation in stain; frequently in a single section the color was patchy.

Because of the variability in the stain of the various blood cells within the vessels of the kidney, we reviewed the literature for glycogen studies on blood and bone marrow in leukemia; marked differences in the loci of positive results are indicated.[7]

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4. Compare figure *2 C* shown in the preceding paper on nephrosis.
5. Compare figure *2 G* in the paper on nephrosis.
6. Gibb, R. P., and Stowell, R. E.: Blood **4**:569, 1949.
7. (*a*) Wachstein, M.: Blood **4**:54, 1949. (*b*) Gibb and Stowell.[6]

Patient	Diagnosis	Age, Yr.	Duration of Illness, Mo.	Blood Count on Admission: Hb, Gm. per 100 Cc.	RBC, Millions	WBC, Thousands	Treatment: Sulfonamide Drugs	Blood Transfusion, Ml.	Aminopterin, Mg.	Duration, Days	Other Agents	Response
B. J. T.	Myeloblastic	14	1	7	2.5	140	+	1,000	2	3	Sodium pteroyltriglutamate	.....
R. L. L.	Myelogenous	6	12	6	1.8	141	+	1,920	31.5	37	.....	None
D. M. R.	Myelogenous	8	4	4	1.2	81	..	3,075	32	18	.....	None
C. C.	Myelogenous	3	3	8	2.3	2.6	+	300	7	10	.....	.....
T. J. R.	Lymphoblastic	5	3	6	1.2	101	+	600	4	4	.....	.....
H. A.	Lymphatic	8	3	9	3	8.8	..	1,400	..	?	Urethan	.....
C. F.	Lymphatic	1	4	9	3	44	..	700	..	?	P^{32}, 1 mc.	.....
A. A. S.	Lymphatic	1.25	3	12	3.8	44	..	350	7.5	10	.....	.....
P. L. R.	Lymphatic	10	8	5	2.1	2.4	+	2,285	50	209	.....	Favorable
L. S.	Lymphatic	5	15	3.5	1	1.6	..	3,400	84	468	.....	Favorable
D. R. M.	Aplastic; lymphatic	4	6	3.5	1.3	6.2	+	1,825	7	7	.....	.....

* Hotchkiss technic.
† Gomori silver nitrate-methenamine technic.
‡ Var. stands for variable.

Many workers [8] have reported that the erythrocytes do not carry glycogen. We corroborate the finding of positive stain in red blood cells obtained by Ellis and Payne,[9] who used macrochemical methods, and of Stahl, Horstmann and Hilsnitz,[10] who used iodine as a vital stain. There is a possibility that the deep stain described is due to glycogen liberated by enzymic cleavage [8b] and subsequently diffused. It had been suggested by numerous early workers, quoted by Stahl and associates,[10] that glycogen parallels hemoglobin concentration.

The view that glycogen content increases with the maturity of the blood cells, especially of the myeloid series, in peripheral circulation and bone marrow,[11] is in contrast with our findings in the kidney, where the deepest stain was encountered in the more immature cells of the lymphoblastic and myeloblastic leukemias. Our observations in the kidney are in agreement with those of investigators [12] who reported that as a group the myelogenous leukemias showed more definitely positive results than did the lymphatic leukemias, which we also found more variable in response.

Whatever the exact nature or composition of the basement membrane, as discussed in a previous paper,[1] the loss of the staining capacity at a definite time in the course of this disease is an interesting observation. An exception was L. S., who had been controlled well with aminopterin but died of pneumococcic meningitis. The kidney from this patient was essentially normal; glycogen stains did not show the loss of reaction in basement membrane but rather an increased color, so that the basement membrane looked thickened.

A diagnosis of lymphatic leukemia was made at the necropsy of D. R. M., although aplastic anemia had been entertained in the clinical differential diagnosis.

8. (*a*) Wagner, R.: Blood **2**:235, 1947; (*b*) Arch. Biochem. **11**:249, 1946. (*c*) Bridge, E. M., and Holt, L. E., Jr.: J. Pediat. **27**:299, 1945. (d) van Creveld, S.: Arch. Dis. Childhood **9**:9, 1934. Gibb and Stowell.[6] Wachstein.[7a]

9. Ellis, R. W. B., and Payne, W. W.: Quart. J. Med. **5**:31, 1936.

10. Stahl, R.; Horstmann, and Hilsnitz: Arch. path. Anat. **267**:392, 1925.

11. Wachstein.[7a] Wagner.[8a] Stahl and others.[10]

12. Wagner.[8a] van Creveld.[8d]

of Glycogen in Kidney

Time of Necropsy After Death, Hr.	Kidney Infiltration	Cloudy Swelling	Stain Reactions for Glycogen									
			Basement Membrane		Brush Border		Granules		Blood Cells		Casts	
			H*	G†	H	G	H	G	H	G	H	G
1	±	++++	+	−	++	+	+++	++	−	++Black	+	±
4	−	++	Var.‡	−	+++	++++	+	++	±	++Red	−	+
6	+	++++++	Var.	−	++++	++++	+++	+	±	++Red	+	−
2	−	+++	+	−	+++	−	+	−	+	++Red	+	−
1.5	−	++	+	+	+	+	±	±	−	+Black	+	−
1.5	+	++	+	−	++	+	+	+	+	+Red	−	−
1.5	+	±	Var.	−	Var.	++	−	±	−	+Var.	−	−
4.5	±	±	+	−	+	+	−	−	−	+	−	−
0.75	+	+	++	+	++	++	±	++	−	+	+	+
2	−	+	+++	+	±	−	−	−	−	+	+	−
6	++	+++	Var.	Var.	+	Var.	−	−	−	+Var.	−	−

Kidney sections were practically without reaction for glycogen. Bone marrow studies on this and other patients with aplastic anemia lead one to agree with Stahl [10] that the matrix of the blood may differ from that of the normal individual. Results of histochemical stains for glycogen, nucleic acids and lipids were so pale that one could scarcely see cells. However, when bone marrow had been mixed with normal serum or with a lactalbumin hydrolysate for half an hour in vitro, stain reactions were enhanced. Even after the cells were subsequently washed with saline solution, staining was adequate, which would indicate, perhaps, that the difference was not solely adsorption of protein on the cells but rather that the cells, presented with proper building materials, were capable of incorporating them into the cytoplasm.

SUMMARY

Glycogen was demonstrated histochemically in the proximal convoluted tubule and the ascending limb of the loop of Henle in kidneys of patients who died of leukemia. The reaction was more marked in the kidneys in myelogenous leukemia than in those in lymphatic leukemia.

FATTY LIVER WITH AND WITHOUT NECROSIS

Histological and Biochemical Investigations

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AND

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THE FUNCTIONAL significance of morphological changes in the liver has not been fully clarified. Although fatty metamorphosis and necrosis are common findings in liver disease, the role played by each lesion in producing biochemical changes in the liver and the serum is obscure. In order to study some of these interrelationships, histological and functional alterations as expressed in biochemical findings in serum and liver tissue were correlated in different stages of two types of experimental liver disease, fatty metamorphosis with and without necrosis.

To produce fatty liver with necrosis in rats, carbon tetrachloride was used. Its intraperitoneal administration resulted in reproducible central necrosis and fatty changes. To produce fatty liver without necrosis, dl-ethionine was used. Ethionine (α-amino-γ-ethylmercaptobutyric acid), a metabolite antagonist of methionine, was previously shown to induce fatty infiltration of the liver in female rats.[1] The fatty liver was prevented by methionine and glucose but was not influenced by several other amino acids.

MATERIAL AND METHODS

Female albino rats, 2 to 4 months old, previously fed a well balanced stock diet and weighing 150 to 200 Gm., were used. Twelve hours before the administration of carbon tetrachloride (CCl_4) and ethionine all food was removed and the animals were fasted thereafter throughout the experiment.

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From the Hektoen Institute for Medical Research and the Departments of Pathology, Cook County Hospital and Northwestern University Medical School.

1. Farber, E.; Koch-Weser, D., and Popper, H.: Endocrinology **48**:205, 1951. Farber, E.; Simpson, M. V., and Tarver, H.: J. Biol. Chem. **182**:91, 1950.

Since the food intake is reduced in both carbon tetrachloride and ethionine intoxication, fasting rats have been used throughout. Fasting in rats in contrast to that in mice [2] does not produce significant increase in liver lipids under the experimental conditions used.

The animals were divided into three main groups: (*a*) one of these remained untreated as controls; (*b*) the second received dl-ethionine in aqueous solution (25 mg. per milliliter) after neutralization with sodium carbonate. It was administered intraperitoneally, 75 mg. per 100 Gm. of body weight in three divided doses, 2½ hours apart; (*c*) the third group received one single intraperitoneal injection of carbon tetrachloride (U. S. P.) diluted 1:10 in liquid petrolatum. The dose was 0.3 ml. (0.03 ml. of carbon tetrachloride) per 100 Gm. of body weight. For this group the intraperitoneal injection was utilized because it gave much more uniform results than the subcutaneous injection, to which some animals are very resistant.[3] Animals of the control and carbon tetrachloride groups were killed after 3 hours, and animals of all three groups after 6, 12, 24, 48 and 120 hours.

The following determinations were performed:

1. *Sulfobromophthalein Clearance.*—Thirty minutes before the animal was killed by bleeding from the abdominal aorta, 5 mg. of sulfobromophthalein sodium per 100 Gm. of body weight was administered intraperitoneally. The concentration of the dye in the serum was determined [4] and expressed in milligrams per 100 ml. of serum.

2. *Liver Weight in Grams per 100 Gm. of Initial Body Weight.*—In all the determinations the initial body weight (weight of the animal at the beginning of the fasting period) was used as reference because there was more loss of weight in the group treated with carbon tetrachloride than in the ethionine-treated and control groups.

3. *Total Liver Lipids.*—An aliquot of liver tissue, ground with anhydrous sodium sulfate, was extracted with chloroform and after evaporation of the solvent was reextracted with petroleum benzin U. S. P. The lipid residue was measured gravimetrically after removal of the petroleum benzin. The results were recorded both in grams per 100 Gm. of liver and milligrams per 100 Gm. of initial body weight.

4. *Total Liver Nitrogen.*—This was determined by nesslerization after digestion with concentrated sulfuric acid. This determination was performed in the 12, 24, 48 and 120 hour groups and recorded in milligrams per 100 Gm. of initial body weight.

5. *Alkaline Phosphatase in Liver.*—Aliquots of liver kept frozen until used were homogenized in an all-glass homogenizer.[5] Alkaline phosphatase was determined [6] using B-glycerophosphate as substrate. The activity of this enzyme was recorded in micromols (μM) per hour per 100 Gm. of initial body weight.

6. *Esterase in Liver.*—This was determined in the same homogenate with phenylbenzoate as substrate,[7] and its activity was recorded in millimols (mM) per hour per 100 Gm. of initial body weight.

7. *Phospholipids in Serum.*—These were determined as alcohol-ether extractable phosphorus (P)[8] in serum obtained at the time the animals were killed.

8. *Serum Bilirubin.*—This was determined according to Malloy and Evelyn.[9]

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2. Hodge, H. C.; MacLachlan, P. L.; Bloor, W. R.; Stoneburg, C. S.; Oleson, M. C., and Whitehead, R.: J. Biol. Chem. **139**:897, 1941. Stowell, R. E., and Lee, C. S.: Histochemical Studies of Mouse Liver After Single Feeding of Carbon Tetrachloride, Arch. Path. **50**:519 (Nov.) 1950.

3. McCloskey, J. F., and McGehee, E. H.: Effect of Subcutaneous and Intraoral Administration of Carbon Tetrachloride on the Liver of the Rat, Arch. Path. **49**:200 (Feb.) 1950.

4. Casals, J., and Olitsky, P. K.: Proc. Soc. Exper. Biol. & Med. **63**:383, 1946.

5. Potter, V., and Elvehjem, C. A.: J. Biol. Chem. **114**:495, 1936.

6. Bodansky, A.: J. Biol. Chem. **101**:93, 1933.

7. Gomori, G.: J. Lab. & Clin. Med. **34**:275, 1949.

8. Fiske, C. H., and Subbarow, Y.: J. Biol. Chem. **66**:375, 1925.

9. Malloy, H. T., and Evelyn, K. A.: J. Biol. Chem. **119**:481, 1937.

9. *Histological Examinations.*—Frozen sections of liver made from material fixed in solution of formaldehyde U. S. P. diluted 1:10 were stained with sudan IV. Paraffin sections of material fixed in Carnoy's solution were stained with hematoxylin-eosin. Identical sections were stained with methyl green-pyronine, for identification of pentose nucleic acid.[10]

RESULTS

Biochemical Alterations.—There was no abnormal sulfobromophthalein retention in the control group throughout the experiment. Both the carbon tetrachloride and the ethionine-treated group had normal values after six hours but showed dye retention after 12 hours. The highest retention occurred after 48 hours in the ethionine group. Both groups were essentially normal again at 120 hours (table 1).

TABLE 1.—*Means with Standard Deviations of Sulfobromophthalein Retention, Liver Weight and Total Liver Lipids of Fasted Rats, Some Untreated and Some Intoxicated with Carbon Tetrachloride or Ethionine*

Rats	Intoxicant Administered	Hours Elapsed When Rats Were Killed	Sulfobromophthalein Retention, Mg. per 100 Ml. Serum	Liver Weight, Gm. per 100 Gm. Body Weight	Total Liver Lipids	
					Gm. per 100 Gm. Liver	Mg. per 100 Gm. Body Weight
3	0	3	.....	2.95 ± 0.18	5.4 ± 0.30	160 ± 9.8
6	CCl_4	3	.....	2.89 ± 0.17	6.8 ± 0.90	198 ± 9.8
3	0	6	0.04 ± 0.017	3.22 ± 0.56	6.0 ± 0.64	180 ± 24.6
7	CCl_4	6	0.18 ± 0.054	3.17 ± 0.37	7.9 ± 1.05	251 ± 7.7
6	Ethionine	6	0.23 ± 0.102	3.20 ± 0.35	7.3 ± 1.08	235 ± 52.0
4	0	12	0.14 ± 0.095	3.19 ± 0.06	5.9 ± 0.64	189 ± 42.2
9	CCl_4	12	2.27 ± 0.540	3.35 ± 0.31	10.9 ± 1.45	381 ± 65.3
4	Ethionine	12	2.58 ± 0.360	3.00 ± 0.16	10.9 ± 1.95	326 ± 44.2
3	0	24	.....	2.75 ± 0.16	5.3 ± 1.02	145 ± 21.4
7	CCl_4	24	.....	3.89 ± 0.33	11.6 ± 1.70	443 ± 71.7
5	Ethionine	24	.....	3.49 ± 0.29	14.9 ± 4.10	512 ± 118.0
6	0	48	0.12 ± 0.120	2.41 ± 0.33	5.3 ± 0.84	130 ± 32.1
10	CCl_4	48	2.29 ± 0.400	3.67 ± 0.22	12.2 ± 1.83	447 ± 84.4
7	Ethionine	48	4.05 ± 0.980	3.82 ± 0.31	22.8 ± 3.85	819 ± 270.0
3	0	120	0.22 ± 0.140	2.15 ± 0.02	5.7 ± 0.68	123 ± 16.2
5	CCl_4	120	0.62 ± 0.470	2.09 ± 0.28	5.9 ± 2.14	128 ± 66.8
5	Ethionine	120	0.35 ± 0.260	2.47 ± 0.24	7.7 ± 1.85	166 ± 109.0

The wet liver weight decreased in the control group from six hours onward, presumably due to the fasting. In both the intoxicated groups there was a marked rise in total liver weight, reaching a maximum in the carbon tetrachloride group after 24 hours and in the ethionine group after 48 hours (table 1).

The total liver lipids, which remained constant throughout the experiment in the control group, were slightly increased in the carbon tetrachloride group after three hours. After six hours they increased progressively in both groups. The highest values were observed in the ethionine group. After 120 hours the values returned to normal in both groups (table 1). The total nitrogen in the liver showed no significant changes in the experimental groups and decreased equally in all three groups because of fasting (table 2).

No decrease in alkaline phosphatase of the liver was observed in the control group after five days of fasting, although a drop of alkaline phosphatase activity of liver tissue has been reported after seven days of inanition [11] and low protein

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11. Miller, L. L.: J. Biol. Chem. **172**:113, 1948.

diet.[12] The enzyme activity did not change significantly in the ethionine group throughout the experiment. In the carbon tetrachloride group, however, a sharp rise was seen beginning at 12 hours. At 24 and 48 hours the activities were found to be four times as high as those of the controls and the ethionine-treated animals (table 2).

The esterase content of the liver decreased uniformly in controls and in the ethionine group with fasting. The activities in the carbon tetrachloride-treated animals at 12, 24 and 48 hours were significantly lower (less than 50 per cent) than in the other groups (table 2).

TABLE 2.—*Means with Standard Deviations of Total Nitrogen, Esterase and Alkaline Phosphatase in Livers and Bilirubin and Phospholipids in Serum of Fasted Rats, Some Untreated and Some Intoxicated with Carbon Tetrachloride or Ethionine*

Rats	Intoxicant Administered	Hours Elapsed When Rats Were Killed	Total N in Liver, Mg. per 100 Gm. Body Weight	Esterase in Liver, mM per Hr. per 100 Gm. Body Weight	Alkaline Phosphatase in Liver, aM per Hr. per 100 Gm. Body Weight	Bilirubin in Serum, Mg. per 100 Ml.	Phospholipid P in Serum, Mg. per 100 Ml.
3	0	3	.....	5.52 ± 0.41	34.8 ± 1.7	0.70 ± 0	5.45 ± 0.95
6	CCl_4	3	.....	4.69 ± 0.68	38.2 ± 7.3	0.70 ± 0.08	4.67 ± 0.57
3	0	6	101 ± 10.5	6.16 ± 2.18	38.2 ± 13.7	0.50 ± 0	5.60 ± 1.75
7	CCl_4	6	86 ± 27.3	5.18 ± 2.32	42.0 ± 18.5	0.54 ± 0.05	4.40 ± 1.32
6	Ethionine	6	103 ± 21.2	6.43 ± 1.38	16.6 ± 9.7	0.60 ± 0.10	5.00 ± 1.47
4	0	12	80 ± 18.2	6.52 ± 1.30	44.2 ± 9.0	0.58 ± 0.13	4.61 ± 0.59
9	CCl_4	12	84 ± 19.1	3.77 ± 0.86	89.1 ± 21.9	1.58 ± 0.41	7.67 ± 0.94
4	Ethionine	12	90 ± 21.7	6.61 ± 0.42	27.1 ± 10.0	1.40 ± 0.30	5.10 ± 0.66
3	0	24	90 ± 17.2	4.40 ± 0.21	29.4 ± 10.0	0.75 ± 0.05	5.50 ± 1.40
7	CCl_4	24	86 ± 8.3	1.65 ± 0.33	198.1 ± 35.8	2.25 ± 0.61	11.90 ± 1.40
5	Ethionine	24	93 ± 10.9	4.01 ± 0.56	35.9 ± 17.9	1.45 ± 0.24	6.10 ± 2.00
6	0	48	62 ± 9.2	3.00 ± 0.75	38.1 ± 9.1	0.68 ± 0.12	5.10 ± 1.50
10	CCl_4	48	74 ± 5.7	0.98 ± 0.52	205.4 ± 13.9	2.10 ± 0.65	8.40 ± 1.20
7	Ethionine	48	75 ± 23.2	3.78 ± 1.47	39.6 ± 13.6	2.68 ± 0.41	5.80 ± 1.00
3	0	120	51 ± 2.7	2.98 ± 0.36	21.4 ± 4.6	0.75 ± 0.05	4.10 ± 0.80
5	CCl_4	120	58 ± 2.8	1.98 ± 0.44	40.1 ± 13.9	0.80 ± 0.21	4.90 ± 0.66
5	Ethionine	120	62 ± 13.0	2.33 ± 0.57	54.7 ± 24.0	0.80 ± 0.20	3.80 ± 0.33

The serum phospholipids remained equally unchanged at all intervals in controls and ethionine-treated animals, while in the carbon tetrachloride group there was a marked increase in 12 to 48 hours, reaching values three times as high as in the other groups at 24 hours (table 2).

The serum bilirubin, always normal in the controls, increased in both the carbon tetrachloride and the ethionine group 12, 24 and 48 hours after intoxication. The peak was at 24 hours in the former and at 48 hours in the latter (table 2).

Histological Alterations.—In the carbon tetrachloride group the histological sections revealed a slight increase in sudan-stainable lipids as soon as three hours after the intoxication. The fat was localized mainly in the intermediate zone in very small intracellular droplets (fig. 1 *A*). After six hours the fat droplets were larger and occupied the cytoplasm of cells in the intermediate and peripheral zones, without being found in the centrolobular zone to a significant degree (fig. 1 *B*). The only alteration observed in this zone at this early stage was a decrease in pyroninophilia, not accompanied with any other visible sign of liver cell damage.

12. Miller, L. L.: J. Biol. Chem. **186**:253, 1950.

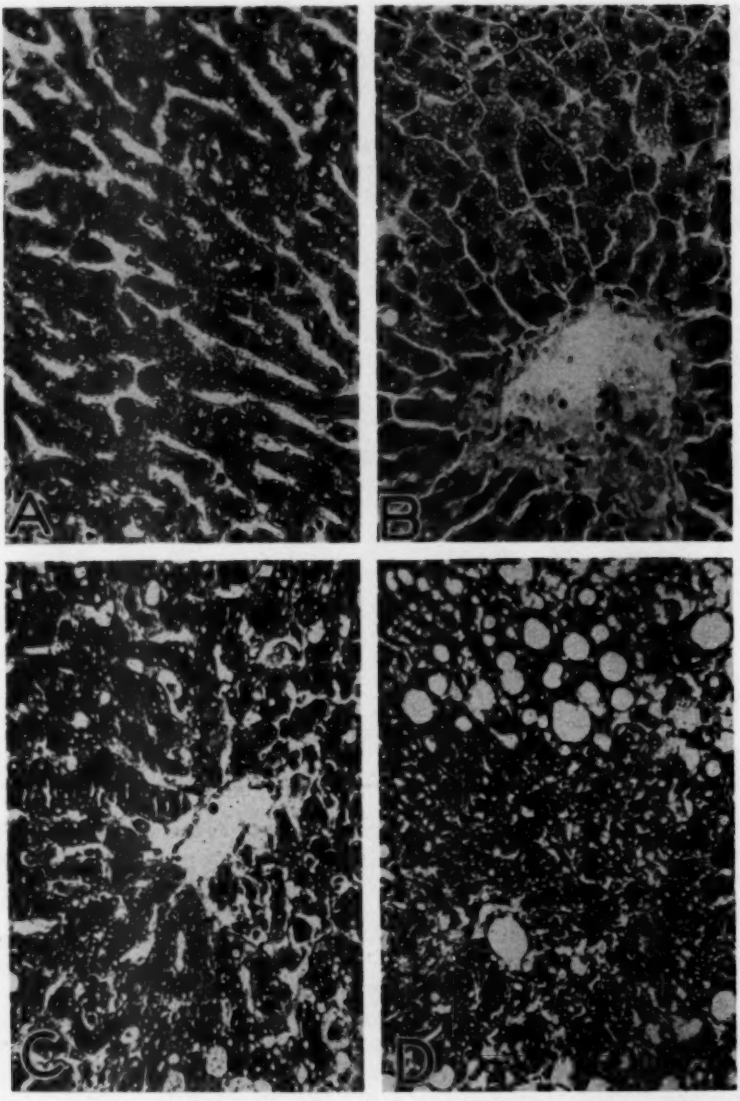

Fig. 1.—Livers of fasting rats treated with carbon tetrachloride; hematoxylin-eosin stain; × 400.

A, 3 hours after intoxication. Few small vacuoles due to fat are noted in the intermediate zone.

B, 6 hours after intoxication. The vacuolation due to fat in the intermediate zone is increased. The liver cells around the central vein are normal.

C, 12 hours after intoxication. Beginning eosinophilic necrosis around the central vein is noted by the smudgy appearance. The vacuolation in the intermediate zone is unchanged.

D, 48 hours after intoxication. There is marked central necrosis. The fat vacuoles in the intermediate zone are large.

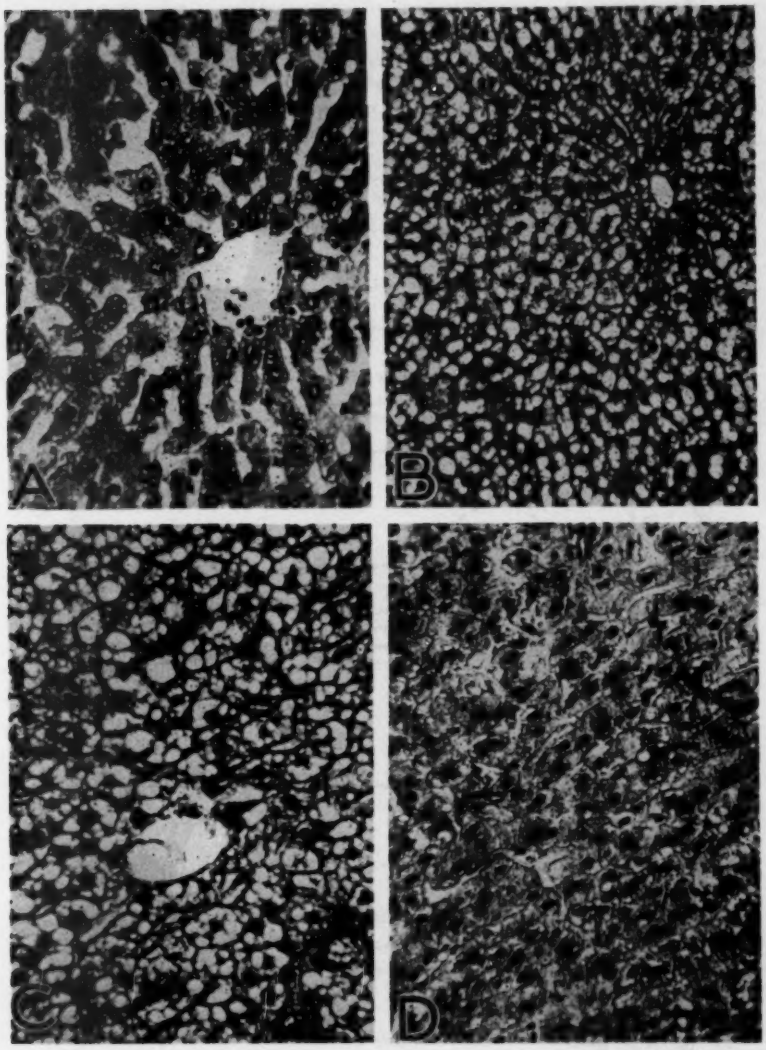

Fig. 2.—Livers of fasting rats treated with ethionine; hematoxylin-eosin stain; × 400.

A, 6 hours after intoxication. Moderate fat vacuolation of the hepatic cells is noted, chiefly in the intermediate zone.

B, 24 hours after intoxication. Medium-sized fat vacuoles crowd hepatic cells in all zones.

C, 48 hours after intoxication. Large fat vacuoles fill the hepatic cells in all zones almost completely.

D, 120 hours after intoxication. Few residual vacuoles are noted.

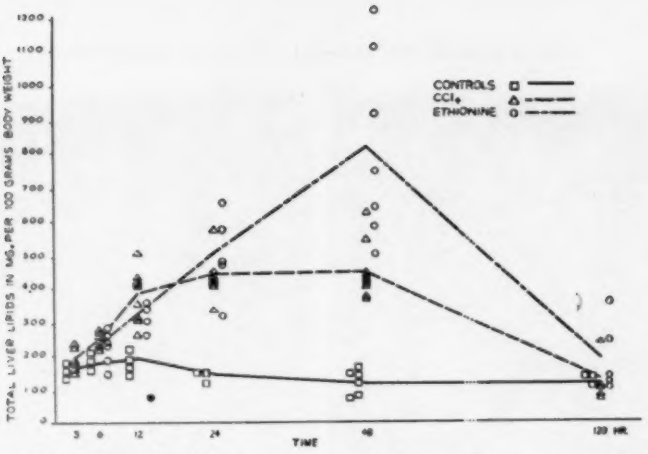

Fig. 3.—Total liver lipids of fasted rats, some of which were untreated and some intoxicated with carbon tetrachloride and ethionine.

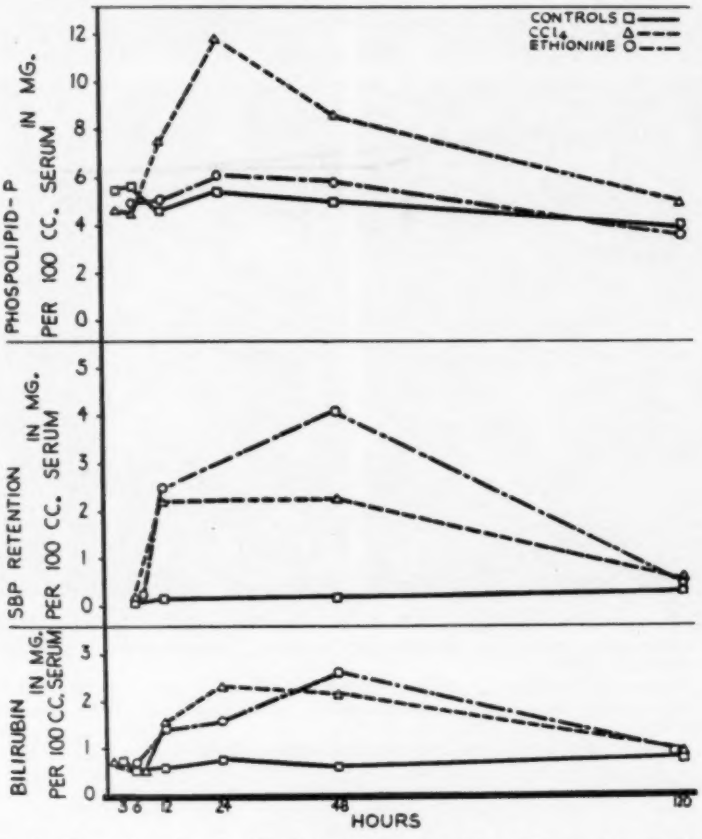

Fig. 4.—Comparison of serum bilirubin, sulfobromophthalein retention and serum phospholipids of fasted rats, some of which were untreated and some intoxicated with carbon tetrachloride and ethionine.

After 12 hours, the fatty infiltration in the intermediate and peripheral zones increased, and the first signs of necrosis were seen in the markedly eosinophilic centrolobular zone. Karyolysis of the liver cells, histiocytic infiltration and Kupffer cell mobilization were noted (fig. 1 *C*). After 24 hours the necrotic area occupied about the central third to half of the lobule. Here the majority of the epithelial cells were destroyed. The remaining cells in the necrotic zone were entirely free of pyroninophilia. Regeneration was not conspicuous in the peripheral zone, whereas after 48 hours intense regenerative activity was observed in that zone,

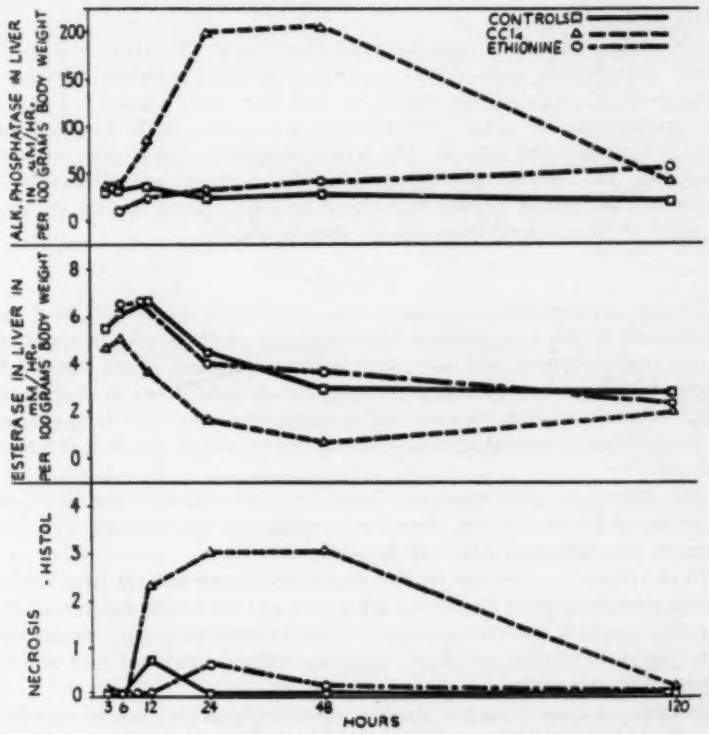

Fig. 5.—Comparison of necrosis, esterase and alkaline phosphatase in the livers of fasted rats, some of which were untreated and some intoxicated with carbon tetrachloride and ethionine.

as indicated by large nuclei with large nucleoli and numerous mitoses in cells with increased cytoplasmic basophilia. Necrosis and fatty changes were essentially the same as after 24 hours (fig. 1 *D*). With the exception of a few small residual areas of necrosis and regeneration, the histological picture of the livers of rats 120 hours after the administration of carbon tetrachloride was normal.

In the ethionine-treated animals the histological appearance of the livers was not different from that of the fasted controls after three hours. After six hours there was very little fat. That present was mainly in the peripheral zone (fig. 2 *A*). The basophilia in cells which contained fat droplets was decreased in degree and

was seen as a small basophilic rim around the nucleus and the fat droplets. No other signs of liver cell damage were noted. After 12 hours the fat droplets increased in size and were also seen in the cells of the intermediate zone. After 24 and 48 hours the cells of the entire lobule were distended with fat droplets (fig. 2 *B* and 2 *C*). The larger fat droplets were usually seen in the peripheral zone. The degree of depletion of pyroninophilia was greatest in areas where the fatty metamorphosis was most marked. No necrotic changes were observed in this entire group. After 120 hours only a few of the ethionine-treated rats showed some residual fatty metamorphosis, while the majority presented a normal histological picture (fig. 2 *D*).

Correlation Between Histological and Biochemical Alterations.—In both the carbon tetrachloride and ethionine groups sulfobromophthalein retention and bilirubinemia accompanied the increase in total liver lipids (figs. 3 and 4). After 48 hours the values from both determinations were higher in the ethionine than in the carbon tetrachloride group. The serum phospholipids rose only in the latter group (fig. 4). The necrotic changes coincided with increase of alkaline phosphatase and decrease of esterase activities in the liver. These enzyme changes did not occur at all in the ethionine-treated animals (fig. 5).

COMMENT

Various investigations on experimental animals and on patients have yielded controversial results as to presence, degree and type of functional alterations caused by fatty metamorphosis and also other structural changes of the liver.[13] Some patients with fatty liver, as proved in biopsy, had minimal if any functional impairment,[14] whereas in other instances, for example, alcoholism with fatty changes as the predominant structural alteration, severe impairment of the liver function can be found.[15]

The older pathologists tried to differentiate fatty infiltration and degeneration by the size of the fat droplets. Small ones supposedly characterized degeneration. However, this difference could not be confirmed.

In an attempt to assess the relative importance of necrosis and fatty infiltration in producing biochemical alterations in the liver and the serum, functional changes were determined in consecutive stages of rapidly developing fatty metamorphosis of the liver with necrosis (produced by carbon tetrachloride) and without necrosis (produced by ethionine).

In ethionine-treated animals the fatty metamorphosis was not accompanied by any other histological sign of liver cell damage (except for decrease in pyroninophilia). Beginning in the periphery the fatty changes reached the maximum

13. Sherlock, S.: J. Path. & Bact. **58**:523, 1946. Hoffbauer, F. W.; Evans, G. T., and Watson, C. J.: M. Clin. North America **29**:363, 1945. Gillman, T., and Gillman, J.: South African J. M. Sc. **10**:53, 1945. Kinsell, L. W.; Weiss, H. A.; Michaelis, G. D.; Shaver, J. S., and Barton, H. C., Jr.: Am. J. Med. **6**:292, 1949. Klatskin, G., and Yesner, R.: J. Clin. Invest. **28**:723, 1949. Popper, H., and Schaffner, F.: Advances Int. Med. **4**:357, 1950.

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15. Davis, W. D., Jr., and Culpepper, W. S.: Ann. Int. Med. **29**:942, 1948. Volwiller, W.; Jones, C. M., and Mallory, T. B.: Gastroenterology **11**:164, 1948.

in intensity and extent after 48 hours, at which time they occupied the whole liver lobule. In a separate publication the significance of the decreased pyroninophilia in those areas has been discussed, together with microchemical determinations which showed no changes in the total amount of nucleic acids in the liver.[16] Of the biochemical properties examined, sulfobromophthalein retention, bilirubinemia and total liver lipids increased parallel with the degree of fatty metamorphosis in the ethionine-treated group. The other findings were in the normal range. The relation of sulfobromophthalein retention and bilirubinemia to the hepatocellular fat accumulation requires some consideration.

Although sulfobromophthalein is partially removed from the blood stream by organs other than the liver,[17] the only significant removal is exerted by the polygonal cells of the liver, as long as these are exposed to the blood flow.[18] Sulfobromophthalein retention is, therefore, considered an indication of either impaired hepatic circulation or altered function of the liver cells or both.[19] The hepatic cells extract it from the blood and excrete it into the bile.[20] It has been shown that rose bengal, which behaves similarly to sulfobromophthalein, is still excreted by the damaged cells of the central zone after carbon tetrachloride intoxication.[21] In this study, in the uncomplicated fatty metamorphosis due to ethionine the sulfobromophthalein retention was about twice as high as that observed in the fatty liver with necrosis, indicating that fat accumulation influences the dye retention more than necrosis does. This is possibly brought about through circulatory impairment due to compression of the sinusoids by the liver cells distended with fat.

The frank increase in bilirubinemia under the same conditions suggests that at least one cause of jaundice can be mechanical interference with bile or blood flow without signs of parenchymal functional impairment, because the biochemical alterations found in the uncomplicated fatty metamorphosis are better explained by a mechanical rather than a functional inability of the liver.

The biochemical changes following carbon tetrachloride intoxication cannot be as easily correlated with one structural alteration, because here fatty metamorphosis, necrosis and regeneration appear in rapid succession and are observed at the same time in different areas of the liver lobule.

All biochemical alterations present in the ethionine-treated animals were also observed in the carbon tetrachloride-treated rats, but to a lesser degree, probably because the fatty changes were less advanced. However, additional changes were found. The increase in alkaline phosphatase activity and the decrease in esterase (phenylbenzoate) activity are probably associated with the necrosis, since they

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occurred only in the carbon tetrachloride-treated rats. The increase of alkaline phosphatase in the liver has been demonstrated microchemically and histochemically with good correlation in active cirrhosis, obstructive jaundice and hepatitis.[22] In necrotic tissue it has been found unchanged [23] or increased.[24] In regenerating liver tissue a rise has always been detected.[25] It is therefore possible that in this experiment the increase of alkaline phosphatase is due to both necrosis and regeneration, although the rise in phosphatase coincides with the appearance of necrosis and precedes that of regeneration. The decrease of liver esterase (acetylcholine) has been established in dogs [26] and rats [27] after carbon tetrachloride intoxication. Until the influence of regeneration on esterase is established, one has, therefore, to assume that its decrease after injection of carbon tetrachloride is due to the necrosis alone. There is no definite explanation for the impressive increase of serum phospholipids which was observed in the carbon tetrachloride-treated rats but not in the ethionine-treated group. This observation suggests that the phospholipid rise in serum is not necessarily due to the same mechanism as the appearance of jaundice, in which case it would have been even more increased in the ethionine-treated group. In the

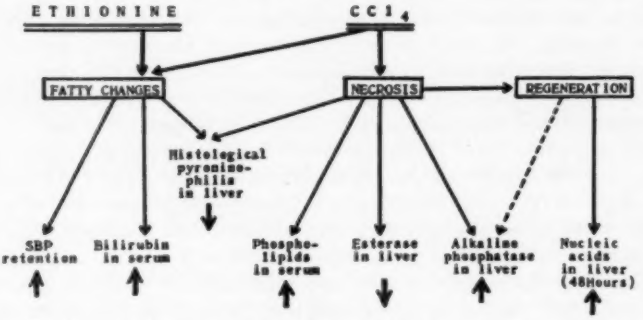

Fig. 6.—Coincidence of histological and biochemical alterations in liver and serum of fasted rats treated with carbon tetrachloride and ethionine.

carbon tetrachloride-treated group the phospholipid rise seems to be rather dependent on or coincident with necrosis. Thus some alterations, at least in the rat, are the result of fatty metamorphosis, whereas others are rather associated with necrosis and/or regeneration. These relations are summarized in figure 6 in which also the

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23. Wachstein, M.: Influence of Dietary Deficiencies and Various Poisons on Histochemical Distribution of Phosphatase in Liver, Arch. Path. **40**:57 (July) 1945.

24. (*a*) Pearson, B.; Novikoff, A., and Morrione, T. G.: Cancer Res. **10**:557, 1950. (*b*) White, J.; Dalton, A. J., and Edwards, J. E.: J. Nat. Cancer Inst. **2**:539, 1942. (*c*) Takamatsu, H.: Tr. Soc. path. jap. **29**:492, 1939. (*d*) Greenstein, J. P.: J. Nat. Cancer Inst. **2**:511, 1942.

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disappearance of the pyroninophilia (discussed in another publication [16]) in ethionine as well as in carbon tetrachloride-treated rats is considered.

The histological alterations appearing in the rat liver at consecutive stages after carbon tetrachloride poisoning seem worth discussing. The fatty changes, localized in the intermediate and peripheral zone throughout the experiment, preceded the central necrosis. But this peripheral fat accumulation does not seem to cause impairment of the blood flow to the central zone and therewith central necrosis, since the same did not develop with even more pronounced fatty changes in the ethionine-treated animals. The impairment of the intrahepatic blood flow, which is a very early result of carbon tetrachloride intoxication, seems to be caused by early swelling of the parenchymal cells.[28] It is possible that some cell damage exists in the central zone even before signs of necrosis are visible, since the cytoplasmic pyroninophilia was reduced in this area in the very early stages. This decrease of basophilia in the prenecrotic phase of dietary liver necrosis [29] and as early as one hour after carbon tetrachloride administration [30] has been reported and seems to be one of the first signs of impending cell damage, even though the lack of parallelism previously reported [31] between cytoplasmic basophilia and chemical analysis allows no definite interpretation at present.

SUMMARY

Sulfobromophthalein clearance, liver weight, total liver lipids, total liver nitrogen, liver alkaline phosphatase and esterase activities and serum phospholipids and bilirubin were determined at consecutive stages in the development of fatty liver without necrosis (induced by ethionine) and fatty liver with necrosis (induced by carbon tetrachloride) in female rats and were correlated with the histological picture.

With ethionine the histologically determined uncomplicated fatty metamorphosis extended over the whole liver lobule and was accompanied by increase in chemically detected total liver lipids, marked sulfobromophthalein retention and hyperbilirubinemia. The latter two were tentatively explained by disturbance in hepatic circulation.

After carbon tetrachloride intoxication the fatty metamorphosis was associated with necrosis and regenerative changes. These were accompanied by sulfobromophthalein retention and jaundice, both less prominent than in the ethionine-treated group, by an increase of hepatic alkaline phosphatase and decrease of hepatic esterase activities, and by a sharp rise of serum phospholipids. The significance of these findings and the appearance of the histological changes at the consecutive stages are discussed.

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FURTHER STUDIES ON THE PATHOGENESIS OF FIBROCYSTIC DISEASE OF THE PANCREAS

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IN A PREVIOUS study [1] we were able to demonstrate that an active extract of secretin could be obtained from the intestine of man at necropsy. In that study we were unable to extract secretin in the only case of fibrocystic disease of the pancreas in which a specimen was available for extraction. From this result and from certain histologic observations [2] it was postulated that a deficiency of secretin in the intestinal mucosa might be responsible for the condition known as fibrocystic disease of the pancreas.[3] Since that time we have had the opportunity to study six more cases of fibrocystic disease of the pancreas at necropsy [4] and to our surprise found that we were able to obtain an active extract of secretin [5] (S_1) in each case. Because of these results the data concerning the original case of fibrocystic disease in which we were unable to extract secretin were reexamined and compared with the data from the subsequent cases in which we were able to extract secretin. The most important difference between the original case and the subsequent six cases was found to be in the length of the portion of duodenum and small intestine available for extraction. The specimen from the original case in which we had failed to obtain secretin was only 51 cm. in length. The specimens from the subsequent six cases from which active extracts had been obtained varied in length from 111 cm. to 562 cm.

In the consideration of these data it was recalled that we were unable to extract active secretin from infants in the neonatal period but that by combining the crude extracts ("A" precipitate) from three premature infants and in another instance from three newborn full-term infants, we had been able to obtain active preparations of secretin (S_1).[5] These facts suggested that secretin was probably present in the original case of fibrocystic disease, but because only a short segment of intestine was

From the Section on Pathologic Anatomy (Dr. Baggenstoss), the Division of Biochemistry (Dr. Power) and the Division of Experimental Medicine (Dr. Grindlay), of the Mayo Clinic and Mayo Foundation.

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4. Five specimens were sent to us by Dr. S. S. Barron, Minneapolis; Dr. J. B. Arey, Philadelphia; Dr. W. A. Newton, Detroit; Dr. C. S. Small, Loma Linda, Calif., and Dr. Gorton Ritchie, Milwaukee.

5. Greengard, H., and Ivy, A. C.: Am. J. Physiol. **124**:427, 1938.

available for extraction, the amount of secretin was so small that it was lost during the process of extraction.

The significant data concerning all cases of fibrocystic disease of the pancreas in which extraction of secretin was performed and the response of the dog to the extracted secretin are shown in tables 1, 2 and 3. These data indicate that there is no

TABLE 1.—*Fibrocystic Disease of Pancreas: General Data*

Case	Age, Mo.	Sex	Duration of Illness, Days	Length of Intestine, Cm.	Postmortem Interval, Hr.
1	16	M	450	51.5	10.0
2	36	M	1,000	316.0	8.0
3	9 wk.	M	42	302.0	2.5
4	3	M	90	111.0	4.5
5	..	..	..	502.0	21.0
6	9½	F	270	332.0	3.0
7	24	F	..	398.0	3.0

TABLE 2.—*Fibrocystic Disease of Pancreas: Extraction of Secretin*

Case	"A" Precipitate: Extraction Delayed, Days	"A" Precipitate: Weight, Gm.	Days Before Alcoholic Extraction	Weight of S_1, Mg.
1	8 hr.	<1	1	12.4
2	8	18	1	100.0
3	40	6	1	20.2
4	62	3	1	13.6
5	81	13	84	50.0
6	62	10	—1	112.0
7	33	26	1	186.0

TABLE 3.—*Fibrocystic Disease of the Pancreas: Response to Extracted Secretin*

Specimen	Dose of S_1, Mg.	Pancreatic Juice: Cc. per 10 Min., Control	Cc. per 10 Min., Test	Nitrogen, Mg. per 100 Cc., Control	Nitrogen, Mg. per 100 Cc., Test	Chlorides, mEq. per L., Control	Chlorides, mEq. per L., Test
1*	12.4	2.8	2.1	276.0	384.0	61.5	71.4
2	10.0	1.1	1.9	100.0	144.0	320.0	252.0
2A†	....	0.5	6.4	.....	70.0	....	34.0
2	58.9	0.9	3.4	234.0	374.4	60.6	44.0
3	10.0	0.7	2.2	60.0	210.0	41.0	49.0
4	13.6	1.3	1.8	296.4	249.6	95.6	79.5
5	12.0	0.1	0.7	379.2	646.8	....	68.8
6	12.0	0.2	1.2	456.0	540.0	66.6	56.7
7	12.0	0.4	2.0	603.6	456.0	77.8	37.5

* No secretory response.
† Secretin, 1 clinical unit, prepared and donated by Eli Lilly & Company.

demonstrable deficiency of secretin in the small intestine in cases of fibrocystic disease of the pancreas.

Since the theory that a deficiency of secretin was responsible for fibrocystic disease of the pancreas has been shown to be untenable, other theories which attempt to explain this disease have been reexamined. Among these theories is that of Farber,[6] who suggested that a disturbance of parasympathetic innervation or an

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autonomic imbalance in nervous control of secretions in the pancreas and in mucous glands might be responsible. According to Farber,[7] the pancreatic lesion of fibrocystic disease can be reproduced in kittens by the injection of pilocarpine over a period of two to eight weeks. These experiments [8] were repeated on kittens, puppies and suckling pigs, but it was not possible to reproduce the lesions of fibrocystic disease of the pancreas or the associated lesions in the lungs in any of these animals.

Another theory holds that deficiency of vitamin A is the etiologic factor in the pathogenesis of fibrocystic disease of the pancreas. This possibility was not investigated in our study because a number of important facts can be marshaled against it: (1) In most cases either there is no squamous metaplasia of the pancreatic ducts or the degree of metaplasia is insufficient to cause obstruction, (2) deficiency of vitamin A of severe degree may not be accompanied by pancreatic lesions [9] and (3) there is no evidence that prenatal deficiency of vitamin A is a causative factor.[10]

Yet another theory of pathogenesis holds that this condition is the result of congenital obstruction (stenosis or atresia of the main pancreatic duct or the duct system). There have been a few cases of fibrocystic disease of the pancreas in which obstruction of the main pancreatic duct has been demonstrated grossly by dissection and probing (Parmelee,[11] case 2; Andersen,[10] case 20). Atresia of the main pancreatic duct (duct of Wirsung) within the papilla has been demonstrated histologically by individual sections by Riniker,[12] and by serial sections by Benoit [13] and Oppenheimer.[14] Stenosis of the main pancreatic duct within the papilla has been demonstrated by serial sections by Kornblith and Otani [15] and by Hurwitt and Arnheim.[16]

In two cases malformation and even absence of intrapancreatic ducts were reported, but serial sections were not made (Gross [17]; Kaufmann and Chamberlin [18]). In view of the fact that both Farber and Andersen and Hodges [19] felt that congenital occlusion of the main ducts is uncommon, we considered it worth while to investigate this aspect of the problem.

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PROCEDURE

Serial sections of the head of the pancreas, including the papilla of Vater, were made in five cases of fibrocystic disease of the pancreas. The cases included three males and two females. The ages varied from 6 days to 3 years. The sections were cut 10 microns in thick-

Fig. 1 (case 7).—Atresia of the duct of Wirsung within the papilla of Vater demonstrated by serial sections. Sections shown are 1, 5, 7 and 10, which correspond to *a*, *b*, *c* and *d*, respectively. The duct of Wirsung breaks up into two groups of branches. The upper group disappears completely within the duodenal musculature in *d* (section 10).

ness, and every other one was mounted and stained with hematoxylin and eosin. The sections mounted were numbered consecutively and studied in series. When it was difficult to follow the interlobular ducts, diagrammatic, magnified sketches were made of each section to facilitate accurate pursuit of the course of the ducts.

RESULTS

This study revealed atresia of the main pancreatic duct (duct of Wirsung) within the papilla of Vater in 1 case (case 7; figs. 1 and 2). In this case the pancreatic duct was followed from its orifice in the duodenum toward the pancreas. It divided into two groups of smaller branches which terminated in connective tissue

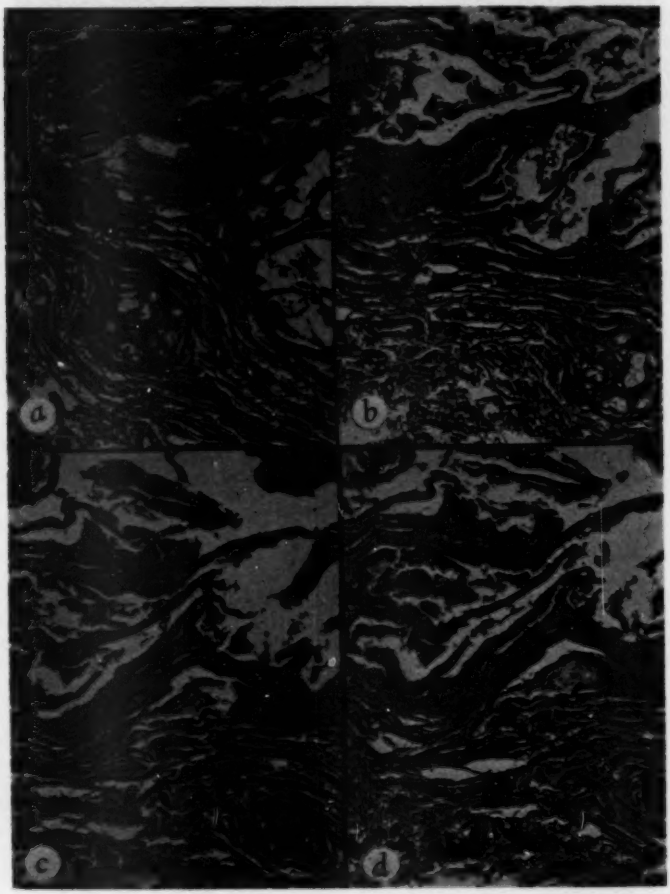

Fig. 2 (case 7).—Continuation of the lower group of branches from figure 1. This group disappears within the duodenal musculature in *d* (section 19).

within the papilla of Vater. There was no evidence of an active inflammatory process in any of these sections.

There was atresia of the main pancreatic duct at its perforation of the duodenal musculature in two cases. In both of these cases atresia of multiple interlobular ducts within the head of the pancreas could also be demonstrated. In neither of

these cases was there any evidence of an active inflammatory process in the sections. In two cases atresia of multiple interlobular ducts alone could be demonstrated (fig. 3). In these cases ducts would commence and terminate in masses of interlobular connective tissue without revealing any connection with groups of acini.

Fig. 3 (case 6).—Atresia of the interlobular ducts within the head of the pancreas demonstrated by serial sections 91, 92, 94, 95, 101 and 103, which correspond to *a*, *b*, *c*, *d*, *e* and *f*, respectively. The right-hand arrow in *a* points to connective tissue where a duct will appear in *b* and disappear in *f*. The left-hand arrow in *a* points to ducts that will disappear in *d*.

In none of these sections was there any evidence of an active inflammatory process at the sites of atresia.

COMMENT

These cases suggest that fibrocystic disease of the pancreas is the result of congenital obstruction of the pancreatic ducts. Although it has been stated that

atresia of the ducts is probably a result rather than a cause of the disease,[20] no evidence for this assumption could be derived from a study of our cases. In each instance of atresia of the interlobular ducts the duct involved arose in a mass of connective tissue as a narrow channel, became dilated and distorted and then gradually became narrower until it disappeared in a mass of fibrous connective tissue. In no instance was there evidence of active inflammation at the site of atresia. A similar chain of events was described and reconstructed into a model by Norris and Tyson [21] from a case of polycystic disease of the pancreas. They concluded that the fundamental defect is the segmentation of epithelial tubules and ducts after their formation in accordance with the normal structure of the organ. The age of the patients, one of them being only 6 days old, also constitutes evidence against the possibility that primary inflammation or reaction to inspissated secretion could have produced the atresia. The fact that extrapancreatic lesions of fibrocystic disease are rare [22] and inconspicuous in infants dying in the newborn period also supports the theory that congenital atresia of the ducts is the basic lesion and that the subsequent lesions of the respiratory tract and other systems are secondary to the pancreatic insufficiency. The evidence derived from the present study supports the theory that congenital atresia of the ducts occurring as an embryologic malformation is the cause of fibrocystic disease of the pancreas.

This study also supports the contention of Hurwitt and Arnheim [16] "that even the most minute gross examination supplemented by a few random sections will not suffice in these cases. We believe that a definitive statement cannot be made unless it is based on a study of serial sections."

If fibrocystic disease of the pancreas is the result of congenital atresia or stenosis of the ducts of the pancreas, the lesions occurring in other organs (intestine, liver and biliary passages, and respiratory tract) remain to be explained. The occurrence of intestinal obstruction from inspissated meconium has been attributed by Farber [6] to the lack of pancreatic juice and digestive action. The frequent occurrence of volvulus and the occasional occurrence of peritoneal bands have been attributed by Andersen and Hodges [19] to the hypermobility of the intestine and peritoneal irritation which may occur after obstruction by meconium. Their interpretations appear to have a sound basis. In the presence of congenital obstruction of the ducts pancreatic digestion would be insufficient to prevent inspissation of meconium and subsequent intestinal obstruction. The intestinal atresia which occurs occasionally in these cases may be interpreted as a second congenital anomaly.

Lesions of the Liver and Biliary Passages.—The lesions of the liver are generally described as local areas of cirrhosis which histologically consist of a concentration of small bile ducts containing eosinophilic concretions and surrounded by fibrous tissue. These lesions have been interpreted as constituting evidence of intrahepatic and partial biliary obstruction. Hypoplasia of the gallbladder and atresia of the cystic duct are other observations frequently made. These lesions can perhaps best be explained as further examples of congenital anomalies occurring concomitantly with obstruction of the pancreatic ducts.

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20. Blackfan and Wolbach.[9] Andersen and Hodges.[19]

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Lesions of the Respiratory Tract.—These lesions consist of emphysema, chronic suppurative bronchitis, bronchiectasis, abscesses and bronchopneumonia. The pulmonary lesions have been attributed to congenital bronchiectasis, to deficiency of vitamin A secondary to steatorrhea, to an abnormality in the bronchial secretion or to an infection of unknown nature which involves both the pancreas and the lungs. The evidence against these theories has been clearly outlined by Andersen and Hodges,[19] and we are in agreement with their conclusion that the pulmonary lesions are secondary to the nutritional disturbances, including deficiency of vitamin A and certain proteins resulting from lack of pancreatic function.

HEREDITARY CONSIDERATIONS

Recent genetic studies [19] have ascertained that cystic fibrosis of the pancreas occurs in families in a manner consistent with the behavior of a hereditary trait. In our opinion this hereditary defect resides in a malformation (atresia or stenosis) of the ducts of the pancreas, and the abnormal secretion of the intestinal glands, biliary tract and occasionally other epithelial glands is dependent on nutritional disturbances resulting from atresia of these pancreatic ducts.

SUMMARY AND CONCLUSIONS

An active extract of secretin (S_1) has been obtained in six of seven cases of fibrocystic disease of the pancreas. The failure to obtain secretin in the first case studied has been attributed to the short length of intestine available for extraction. These results render untenable the theory that a deficiency of secretin in the small intestine is the primary defect in fibrocystic disease of the pancreas.

A study of five cases of fibrocystic disease of the pancreas by serial sections of the head of the pancreas and the papilla of Vater revealed atresia of the main pancreatic duct within the papilla of Vater in 1 case, atresia of the main pancreatic duct at its perforation of the duodenal musculature and atresia of multiple interlobular ducts in two cases and atresia of interlobular ducts alone in two cases.

The evidence derived from the present study supports the theory that congenital atresia of the ducts occurring as an embryologic malformation is the cause of fibrocystic disease of the pancreas.

OSSEOUS CHANGES IN CONGENITAL BILIARY STENOSIS

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ALTHOUGH many examples of anomalies of the bile ducts had been reported, no attention had been given to the causal relationship between biliary atresia and rickets until Gerstenberger's [1] clinical description of three cases of liver disease with associated rickets. Two of his patients had congenital biliary stenosis. He introduced the term "rachitis hepatica" to designate the syndrome of rickets due to liver disease.

Kirshbaum [2] and Altschule [3] found evidence of rickets at the autopsies of infants with biliary stenosis, but their reports did not include histologic descriptions. The first full pathologic description of hepatic rickets was presented by Thoenes and Gruson.[4] Later Teuber [5] reported another case, giving complete gross and microscopic descriptions. Although only a few other references to the concomitance of these conditions were found in the investigative literature,[6] it is well known clinically and is discussed briefly in textbooks of pediatrics.[7]

PHYSIOLOGIC CONSIDERATIONS

The cause of rickets is a deficiency of vitamin D. This vitamin is obtained either orally or by the action of ultraviolet rays on its precursors in the skin. Vitamin D apparently acts by facilitating intestinal absorption of calcium or phosphates, or both. As a fat-soluble material, vitamin D is not absorbed in the absence of bile salts. There is some evidence that hepatic damage may have an additional adverse effect on the utilization of vitamin D, even if intestinal absorption of fats, as determined by fat excretion studies, is not greatly impaired.[8]

From the Department of Pathology, University of Michigan.

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MATERIAL

As of July 1950, 13 cases of congenital biliary atresia were included in the autopsy series at the University of Michigan Hospital. The ages of the infants concerned ranged from 6½ hours to 8 months. In seven cases specimens through the costochondral junction were available. In five of the remaining six cases portions of other bones had been taken. Sections were prepared, after the specimens had been decalcified by the formic acid method, and were stained with hematoxylin and eosin.

CASE SUMMARIES

The cases are presented in ascending order according to the age of the patients at death..

CASE 1.—A girl (A-199-AI) was born, Jan. 1, 1931, after seven months' gestation. Onset of respiration was delayed for five minutes after birth, and breathing was labored. There was marked ascites but no jaundice. The patient lived 6½ hours. The autopsy findings were: partial agenesia of intrahepatic bile ducts; congenital intralobular cirrhosis; congenital heart disease (redundant plica semilunaris obstructing foramen ovale, right ventricular hypertrophy); congenital cystic kidneys; birth trauma (meningeal and subtentorial hemorrhages); erythroblastosis fetalis. No extrahepatic biliary anomalies were found, and there was no icterus.

The only bone available for examination was from vertebral bodies. Sections showed no increase in osteoid tissue, and the cartilage cells in the proliferating area were small and arranged in short cords.

CASE 2.—A boy (A-49-AW), born at term in May 1944, was admitted to the hospital at 3 weeks of age because of feeding difficulties and diarrhea. He was given vitamin supplements before and after admission. His skin was of normal color, there was no history of acholic stools or dark urine, nor were these signs of icterus observed in the hospital. Treatment consisted of blood transfusions, sulfonamide medication and controlled diet. Diarrhea persisted, and the patient died at 6 weeks of age. The autopsy findings were hypoplasia and stenosis of the common bile duct, obstructive jaundice and perilobular cirrhosis of the liver.

Sections of the costochondral junction were not available. Bone from the sternum and from a rib showed well formed trabeculae with occasional small areas of osteoid tissue.

CASE 3.—A full-term boy (A-30-AS), born in June 1940, had icterus of variable degree, acholic stools and dark-colored urine from birth. The only abnormal physical finding was jaundice. A laparotomy was performed, and since neither gallbladder nor extrahepatic ducts could be found, a drain was placed in the hepatic duct. Abundant bile flowed from the drain until death. The abdomen became distended, and 11 days after the operation the wound opened, with herniation of small bowel and omentum. The infant died two days after this at the age of 7 weeks. Ascorbic acid, vitamins D and K, and bile salts had been administered preoperatively and postoperatively. The autopsy findings were: congenital absence of the common and cystic bile ducts and gallbladder; jaundice and biliary cirrhosis with early atrophic cirrhosis; hepaticotomy; acute purulent peritonitis.

There was no irregularity of replacement of cartilage at the costochondral junction. The cartilage cells were regularly arranged in cords. Normal amounts of osteoid tissue were present about the trabeculae. The proliferating cells of the vertebral cartilage were small and in short cords, and calcification of these cells was normal.

CASE 4.—A girl (A-204-AT), born at full term in September 1941, was admitted to the University Hospital subsequent to an exploration of the extrahepatic bile ducts elsewhere. She had been icteric and the stools acholic since birth. The laparotomy had disclosed atresia of the bile ducts. A second laparotomy was done, but no reconstruction was possible. She was treated with ascorbic acid, vitamins D and K and transfusions. She died at 3 months of age. The autopsy findings were: aplasia of the common bile duct; marked hypoplasia of the gallbladder; advanced biliary cirrhosis; chronic icterus; laparotomies; infection and gangrene of the operative field; acute fibrinopurulent peritonitis on chronic adhesions.

The only sections of bone available were from vertebrae. There was a moderate amount of osteoid tissue. The cartilage cells were small, heavily calcified and arranged in short cords.

CASE 5.—A full term girl (A-237-AZ), born in July 1947, became jaundiced three weeks after birth. The urine was consistently dark, and the stools varied from clay-colored to a normal color. Cod liver oil had been included in her diet. The icterus was the only significant physical abnormality. A roentgenogram of the chest showed no skeletal deformity. A laparotomy was done and an attempt made to establish drainage from the hepatic ducts. A soluble mixture of vitamins was administered. The patient died 12 days after the operation, at the age of 5 months. The autopsy findings were congenital atresia of the extrahepatic biliary tract, obstructive jaundice and biliary cirrhosis.

There was heavy calcification of the cartilage cells of a vertebral body, and the cells were small and arranged in short cords. There was a small amount of osteoid tissue.

CASE 6.—A girl (A-206-AO), born at term in May 1936, became jaundiced at 3 weeks of age. The stools were acholic and the urine dark. She had not received supplemental vitamin D. The only physical abnormality was the icterus. She was treated with vitamins and transfusions. Operation was not attempted. The patient was 5 months old at the time of death. The autopsy findings were: congenital atresia of the gallbladder and of the hepatic, cystic and common ducts; cirrhosis of the liver; obstructive jaundice; acute purulent bronchitis and bronchopneumonia.

There was abundant calcification of the cartilage matrix of the vertebrae. The cells were small and in short cords. The osteoid tissue was slightly increased in amount.

CASE 7.—A boy (A-230-BB), born at full term in September 1948, was jaundiced at birth. The jaundice varied in intensity, but the stools were constantly acholic and the urine dark. Physical examination revealed icterus and hepatomegaly. At 6 months of age, laparotomy disclosed a congenital biliary atresia which could not be repaired. The autopsy findings were: congenital absence of the gallbladder and the hepatic, cystic and common ducts; cirrhosis of the liver; obstructive jaundice; acute purulent bronchitis and bronchopneumonia.

Sections of costochondral junctions showed a regular calcifying zone with normal destruction of cartilage cells. The cells were regularly arranged in cords. The amount of osteoid tissue was not increased. The cartilage cells of a vertebral body were small, arranged in short cords and well calcified.

CASE 8.—A boy (A-250-AW), born at term in March 1944, became jaundiced at 3 weeks of age. At that time he also bled from the umbilicus. The stools were acholic, and the urine was dark. On physical examination marked icterus, splenomegaly and softening of the occipital bones were found. Roentgenograms of the long bones revealed the changes of rickets. At operation, bile was found in the gallbladder, but no common duct could be demonstrated. The patient was 6 months old at the time of death. The autopsy findings were: congenital biliary atresia, probably of the common duct near the point of origin; death five days after exploratory laparotomy in which the bile was drained from the gallbladder; early biliary cirrhosis; obstructive icterus; portal obstruction; dilatation of retroperitoneal, hemorrhoidal and esophageal veins; rickets.

In sections of a costochondral junction the zone of calcification of cartilage was irregular, the cartilage cords were arranged in a disorderly fashion, and capillary buds grew into the cartilage plate. Because of failure of destruction of cartilage cells, areas of cartilage projected into the marrow spaces. The trabeculae showed wide zones of osteoid tissue. In a vertebral body there was abundant osteoid, the cords of cartilage cells were increased in length, the growing cells were larger than normal, and calcification was defective.

CASE 9.—A girl (A-174-AS), born at term in May 1940, became jaundiced on the fourth day of life. The mother had been treated for syphilis, and the child was given heavy metal therapy. Adequate vitamin supplements were added to her diet. At the time of admission to the University Hospital, icterus and thickening of the costochondral junctions were the important physical findings. The Kahn test was negative. While in the hospital the infant was treated with vitamin K, synthetic vitamin D and bile salts. She died at 6 months of age. The autopsy findings were: congenital atresia of the hepatic ducts; cirrhosis of the liver; obstructive icterus; hemorrhages into lungs, skin and periosteum.

The cartilage plate of the costochondral junction was irregular. Destruction of cartilage cells was incomplete, and capillary buds had grown into the cartilage. Trabeculae composed

almost entirely of osteoid tissue appeared in some of the spaces thus formed. In the vertebral bodies the thickened trabeculae were covered by heavy zones of osteoid tissue. The cords of proliferating cartilage were long, and the cells were large.

CASE 10.—A girl (A-211-AY) was born at term in November 1945. The delivery was precipitous. She was icteric at birth. Feeding was by breast; vitamins were not given. At the time of admission to the hospital the infant was icteric and there was definite widening of the costochondral junctions. No operations were attempted. She was treated with penicillin, a mixture of soluble vitamins, and ascorbic acid. Death occurred at 7 months. The autopsy findings were: congenital atresia of the common bile duct; obstructive icterus; advanced biliary cirrhosis; acute mucopurulent laryngobronchitis and bronchiolitis; fibrinopurulent lobular pneumonia.

In longitudinal sections through the costochondral junction, moderate irregularity of calcification of the cartilage and several small capillary buds growing into the cartilage plate were found. The proliferating cartilage cells were in random arrangement, and small clumps of cartilage protruded into the marrow spaces. There was a moderately thick osteoid zone about the trabeculae. In a vertebral body the columns of cartilage cells were increased in length and the proliferating cells were larger than is normal. Calcification was defective.

CASE 11.—A full term boy (A-323-AZ) was jaundiced at birth. The stools were light but not acholic, and the urine was dark. The diet was considered adequate. On admission the pertinent physical findings were jaundice and a deformity of the costochondral junctions which suggested scurvy to the examiner. The biliary system was explored and found to be irreparably distorted. One month after operation the patient died; he was then 8 months old. The autopsy findings were congenital biliary atresia; biliary cirrhosis; obstructive icterus; rachitis; localized subacute fibrinous peritonitis; ascites.

Microscopic examination of the costochondral junction showed marked irregularity in the calcification of cartilage and incomplete and irregular destruction of cartilage cells. Large masses of cartilage protruded into the marrow spaces, and large capillary "bushes" extended into the cartilage plate. The columns of proliferating cells were distorted. There was abundant osteoid tissue about the trabeculae. The vertebral cartilage also had elongated cords of large, poorly calcified cells and increased amounts of osteoid tissue on the trabeculae.

CASE 12.—A girl (A-288-AZ), born by breech delivery after nine months' gestation, became jaundiced during the first week of life. Her diet was considered adequate. When admitted to the hospital at 7 months of age, she showed icterus and prominent beading of the costochondral junctions. She was treated with a mixture of soluble vitamins and with penicillin and sulfonamides. Operation was not attempted, and she died at 8 months of age. The autopsy findings were congenital atresia of the extrahepatic bile ducts, obstructive jaundice and advanced biliary cirrhosis. "Pea-sized nodules along all costochondral junctions" were described in the protocol.

In a costochondral junction the calcification of the cartilage was markedly irregular. There were irregularities also in the pattern of the cartilage cords and ingrowths of capillary buds in the cartilage plate. The trabeculae were slender, and some appeared to consist entirely of osteoid tissue. The vertebral trabeculae were surrounded by wide bands of osteoid tissue; the cartilage cords were elongated, and the proliferating cartilage cells were enlarged.

COMPILATION OF CLINICAL MANIFESTATIONS

The 12 patients used in this study ranged in age from 6½ hours to 8 months at the time of death. The youngest was a premature infant who died of respiratory failure. Partial agenesis of intrahepatic bile ducts and other congenital anomalies were found at autopsy, but there was no clinical evidence of these abnormalities. In the case of a 6 week old infant brought to the hospital because of a feeding problem, no history of jaundice was obtained, but complete stenosis of the common bile duct was found at autopsy. The remaining 10 patients had icterus as a presenting complaint. Five had been jaundiced since birth, and in none did the icterus appear later than the third week of life. All these patients had dark-colored urine and light-colored to acholic stools.

A clinical diagnosis of rickets was made in five cases, either by observation of enlarged costochondral junctions, by roentgenograms of the long bones or, in one case, by unusual softness of the occipital bone. Rickets was not apparent clinically in infants under 6 months of age.

One infant had parents who had been treated for syphilis, and a diagnosis of congenital syphilis had been made by roentgenography of the long bones. Antisyphilitic heavy metal therapy had been instituted before admission to this hospital. The Kahn reaction of the infant's blood was negative.

Exploratory laparotomies were performed on five of the infants. In each of these an anomaly of the biliary tract, not amenable to surgical correction, was found.

COMPILATION OF GROSS AUTOPSY FINDINGS

In one infant, the youngest of the series, the biliary obstruction was entirely intrahepatic. There were no evidences of rickets, jaundice or biliary cirrhosis. Congenital anomalies of the heart and the kidneys were found, and death was attributed to respiratory failure. The remaining subjects exhibited frank icterus at death and had biliary cirrhosis of varying degree. Bile ducts were found to be completely occluded somewhere along their course.

Gross evidences of skeletal change were present in three of the older infants, each of whom had a typical rachitic rosary.

COMPILATION OF MICROSCOPIC OBSERVATIONS

The earliest skeletal changes were found in two infants dying at 6 months of age. Sections of costochondral junctions from these infants showed striking irregularity of the cartilage cords, decreased calcification of the cartilaginous matrix, irregular destruction of cartilage cells, capillary buds growing into the cartilage plate and large amounts of osteoid tissue about the trabeculae (figs. 1 and 2). Vertebral trabeculae, also, were covered by abundant osteoid tissue, and the proliferating cartilage cells were large, arranged in long cords and only slightly calcified (fig. 3). The costochondral junction from a 7 month old infant showed similar changes. The deficiency of calcification was a prominent feature (fig. 4). The changes in the vertebral bodies were similar to those of the younger patients. The most advanced rachitic change was found in bones from infants who survived for eight months. In the costochondral junctions the proliferating cells were large and had no semblance of orderly arrangement. Calcification was slight, and destruction of cartilage cells was irregular, leaving many large areas of cartilage projecting into the marrow spaces. Numerous large capillary bushes penetrated the cartilage plate. Osteoid tissue was abundant about the trabeculae (fig. 5). The vertebral changes were similar to those in the less advanced cases (fig. 6).

In seven cases no skeletal changes were found. These represent the young group; the oldest of these patients died at 6 months. Sections of costochondral junctions were available from only two of these patients. The margins of the cartilage plates were sharp and regular. The cartilage cells were arranged in straight cords. Calcification was normal in amount, and there was no increase in osteoid tissue about the trabeculae. The cartilage cells of the vertebral bodies were small,

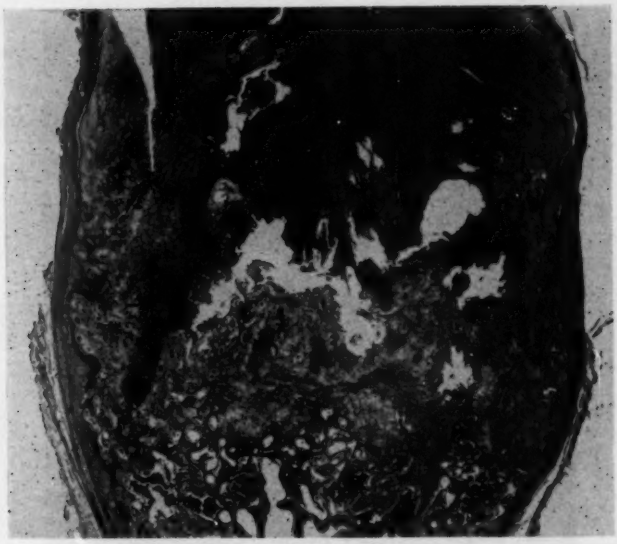

Fig. 1 (case 8; age of patient, 6 months).—Costochondral junction showing typical rachitic changes. Hematoxylin and eosin stain; × 10.

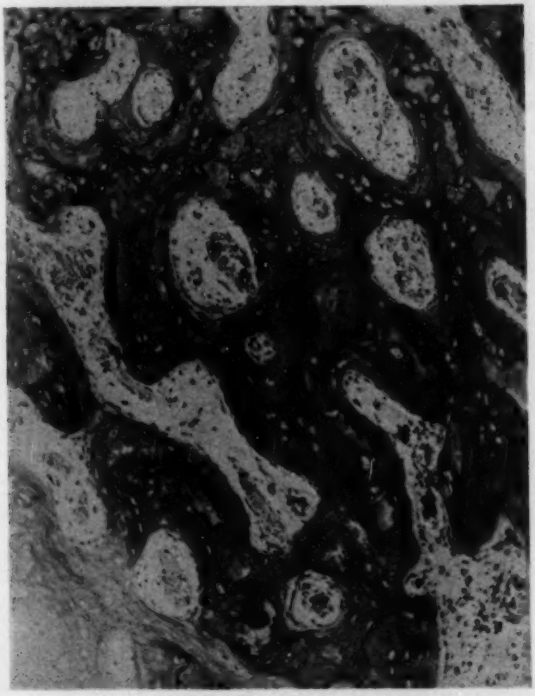

Fig. 2 (case 9; age of patient, 6 months).—Trabeculae of a rib near the costochondral junction, showing abundant osteoid tissue. Hematoxylin and eosin stain; × 150.

Fig. 3 (case 8).—The cartilage plate of a vertebral body showing long cords of large cartilage cells. Osteoid tissue is abundant on the trabeculae. Hematoxylin and eosin stain; × 150.

Fig. 4 (case 10; age of patient, 7 months).—Typical rachitic deformities of a costochondral junction with marked deficiency of calcification. Hematoxylin and eosin stain; × 10.

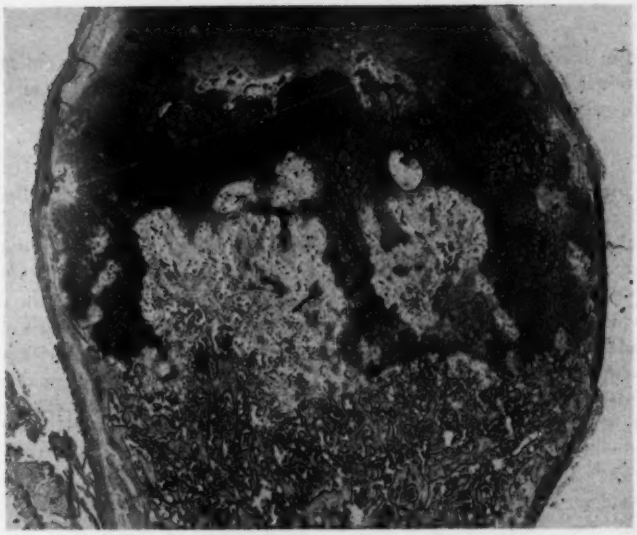

Fig. 5 (case 11; age of patient, 8 months).—Marked rachitic changes of a costochondral junction. Hematoxylin and eosin stain; × 10.

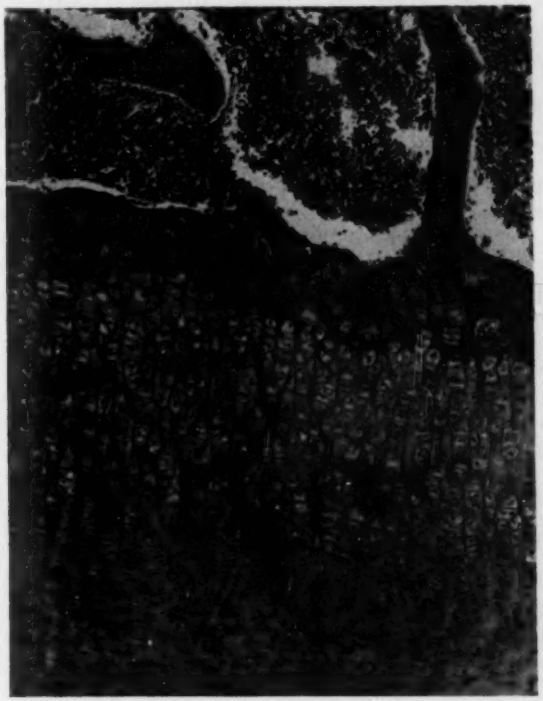

Fig. 6 (case 12; age of patient, 8 months).—The cartilage plate of a vertebral body showing long cords of large cartilage cells. Hematoxylin and eosin stain; × 150.

arranged in short cords, and were well calcified. Osteoid tissue was not increased. The vertebral bodies of the other patients in this group showed the same pattern and were considered to be nonrachitic.

SUMMARY

The osseous changes occurring in the costochondral junctions of older infants dying of congenital biliary atresia are similar to the changes described by Park[9] as typical lesions of rickets. In five of 12 cases in the present series, positive evidences of rickets were demonstrated, and rickets could be ruled out definitely in only two of the remaining seven. In five cases there were no changes in the vertebral bodies which would indicate rickets. In five cases a clinical diagnosis of rickets had been made and was confirmed by microscopic demonstration of skeletal lesions. That rachitis was not shown in the other seven appears to have been due largely to the short period of survival, for 6 months was the earliest age at which evidence of rickets was detected.

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PRODUCTION AND PATHOGENESIS OF PARABIOTIC HYPERTENSION IN THE RAT

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THE EFFECT of the parabiotic state on the development of arterial hypertension in the rat has been studied in several ways. Jeffers, Lindauer, Twaddle and Wolferth [1] reported that following the extirpation of three of the four kidneys in a parabiotic pair of rats, hypertension developed in the totally nephrectomized twin. Grollman and Rule [2] amplified this by showing that bilateral nephrectomy of one of the pair produced hypertension in that animal. When hypertension is induced in one of the parabionts by unilateral perinephritis, according to Grollman and Rule,[2] hypertension develops in the perinephritic rat but not in the attached parabiont. From this it is concluded that either the pressor substance released from the perinephritic kidney does not cross the capillary anastomosis between the pair, or if it does it is rapidly destroyed. Braun-Menendez and von Euler,[3] on the other hand, found that if hypertension is first induced in a rat by unilateral perinephritis and the animal is then placed in parabiosis with a normal animal, the blood pressure of the former declines within a few days to normal levels, but may be reinduced by severing the formerly hypertensive animal from the normal parabiont. When such a hypertensive animal is placed in parabiosis with a totally nephrectomized rat or another hypertensive animal, however, the elevated blood pressure persists in the former.[4] This would suggest that the pressor substance does cross and that a normal animal, but not one that is either nephrectomized or hypertensive, can destroy it rapidly.

The experiments reported to date have always dealt with hypertension induced in one of a parabiotic pair by means known to cause hypertension in the single rat, and the possibility that parabiosis per se might induce an elevation of blood pressure apparently has not been considered. Certain of our observations on hypertension produced in one of a parabiotic pair by desoxycorticosterone acetate treatment of one of them [5] suggested that perhaps parabiosis alone might induce

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2. Grollman, A., and Rule, C.: Am. J. Physiol. **138**:587, 1943.

3. Braun-Menendez, E., and von Euler, U. S.: Rev. Soc. argent. biol. **24**:355, 1948.

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hypertension. The present communication reports the results of an experiment designed to test the validity of this hypothesis.

METHODS AND MATERIALS

Eighty female albino rats of the Holtzman strain, weighing 55 to 80 Gm., in each case litter mates matched to within 5 Gm. of body weight, were paired and placed in parabiosis according to the technique of Bunster and Meyer.[6] Since we wished to compare the response of these rats with that of a previous series which had been treated with desoxycorticosterone acetate and sodium chloride,[5] they received a 1 per cent sodium chloride solution to drink and a purchased laboratory chow [7] ad libitum.

The blood pressure of each rat was measured once weekly without anesthetization, the tail plethysmograph method of Williams, Harrison and Grollman [8] as modified by Sobin [9] being used. In each instance the average of four successive readings which agreed to within 5 mm. Hg was taken as representative of the blood pressure of the animal. A pressure of 150 mm. Hg was arbitrarily considered to be the lower limit of hypertension, although pressures above 135 mm. Hg are very rarely encountered by us in normal rats.

Twenty-eight pairs survived for two weeks or longer, but only the 20 pairs which survived for four weeks or longer are presented in detail in the present communication. On these, autopsies were made at intervals of from 29 to 45 days. At autopsy the animals of a pair were carefully dissected apart and weighed separately, and from each the pituitary gland, the adrenal gland, the thymus, the cardiac ventricles, the kidneys and segments of the pancreas and mesentery were excised and placed in Bouin's fixative for weighing and subsequent microscopic examination.

RESULTS

Hypertension, beginning as early as eight days after the start of parabiosis or as late as the fourth week postoperatively, developed in one of the partners in 12 of the 28 pairs which survived two weeks or longer. In no instance was hypertension observed in both members of a parabiotic pair. However, in most cases marked hypotension was manifested by the co-twin of the rat which later became hypertensive, either just before or at the time when hypertension first became manifest. So consistent was this that when the blood pressure of a parabiotic rat dropped to hypotensive levels for two successive readings the attached parabiont always became hypertensive. Eight of the 28 pairs died at various intervals between the fourteenth and twenty-eighth days of parabiosis. In four of these pairs, one of the partners had become hypertensive, and in an equal number both partners remained normotensive. The remaining 20 pairs, of which 12 were normotensive while 8 consisted of a normotensive-hypertensive partnership, were killed at intervals between the twenty-ninth and the forty-fifth postoperative day. Inasmuch as we were primarily interested in the cardiovascular pathologic changes which might accompany the hypertensive disease, it was felt that uniformity of time of parabiosis was of less importance than uniformity of duration of hypertension. In some cases the hypertensive partner appeared to be in acute distress by the twenty-ninth day postoperatively and nodules, which at autopsy proved to be periarteritic nodules of the superior mesenteric artery and its branches, could be detected by palpation; whereas in other pairs hypertension had barely begun to develop by this time and,

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7. The chow used was made by the Ralston Purina Company, St. Louis.

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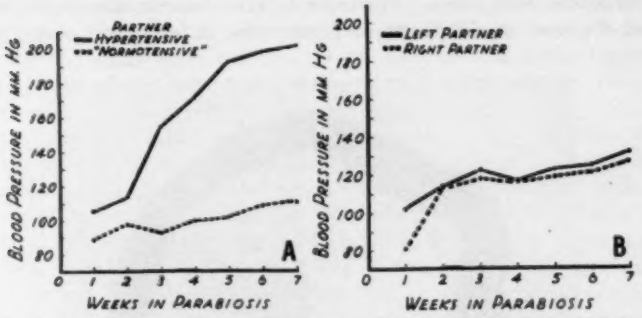

Fig. 1.—*A*, average blood pressures of eight pairs of parabiotic rats. In each of these pairs marked hypertension developed in one of the partners. The attached parabionts are identified as "normotensive" because their blood pressures, though often normal, were with equal frequency hypotensive.

B, average blood pressures of 12 pairs of parabiotic rats. In these pairs the pressures of both parabionts remained within normal limits.

Fig. 2.—A pair of parabiotic rats which received a 1 per cent solution of sodium chloride to drink. Note the prominent edema of the cheeks, the thoracic and the abdominal regions in the partner at the left. The partner at the right is entirely normal in appearance. Sixth day of parabiosis.

doubtless, lesions were absent. Therefore, hypertensive rats were killed only after they had displayed an elevated blood pressure for at least three weeks, at which

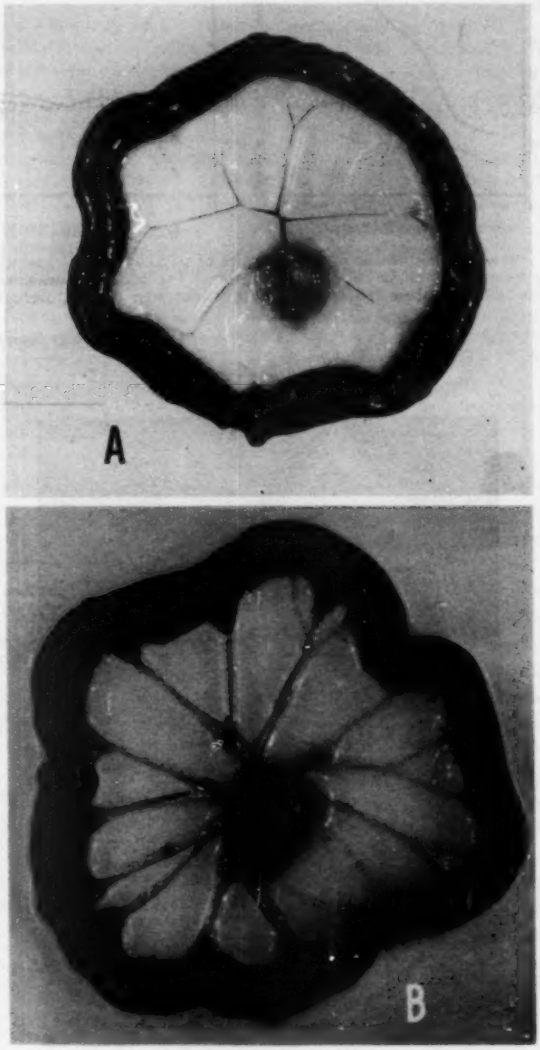

Fig. 3.—*A*, mesenteric loop from the normotensive member of a pair of rats in parabiosis for 36 days. Note the normal appearance of the arterial branches.

B, mesenteric loop from the hypertensive co-twin of the above rat. Note the prominent nodules along the course of the mesenteric vessels, typical of periarteritis nodosa.

time one or more pairs of normotensive rats in parabiosis for the same number of days were killed.

The blood pressure of a rat once it attained hypertensive levels continued to rise progressively and steadily. A comparison between the blood pressures of the 12 normotensive parabiotic pairs and those of the eight pairs in which hypertension developed in one of the partners is given in figure 1. Not only was the peculiar unilateral sensitivity to hypertensive disease apparent, but in many cases there occurred a massive edema of one member of the pair (fig. 2). The time of onset of this varied from four to 30 days postoperatively, although it was much more prone to occur during the first week postoperatively than thereafter. Many of the deaths in the pairs, which were thereby eliminated from our experimental series, were due to the development of massive edema, hydrothorax and even ascites in one of the partners. In the acute form this could readily be reversed by substituting tap water for the saline solution for 24 hours, after which it might or might not recur on return to the saline regimen. This manifestation was not correlated in

The Body and Organ Weights, Together with the Incidence and the Severity of the Cardiovascular Lesions, of Normotensive and Hypertensive Parabiotic Rats

Data Compared	Group: Normotensive-Normotensive — Left Partner	Normotensive-Normotensive — Right Partner	Hypertensive-Normotensive — Hypertensive Partner	Hypertensive-Normotensive — Normotensive Partner
Number of rats	12	12	8	8
Body weight, Initial, Gm.	67 ± 2	68 ± 2	65 ± 3	67 ± 3
Body weight, Final, Gm.	146 ± 7	141 ± 10	143 ± 11	150 ± 8
Heart weight, Gm.	.519 ± .023	.485 ± .019	.706 ± .0388†	.477 ± .019
Heart weight, % B. W.*	.361 ± .017	.370 ± .033	.506 ± .0308	.316 ± .011
Kidney weight, Gm.	1.476 ± .104	1.450 ± .071	1.719 ± .1008	1.507 ± .100
Kidney weight, % B. W.*	1.015 ± .051	1.096 ± .101	1.233 ± .0798	1.005 ± .019
Adrenal weight, mg.	43.9 ± 9.2	46.8 ± 4.0	58.0 ± 4.08	51.3 ± 5.0
Thymus weight, Gm.	.227 ± .031	.214 ± .025	.179 ± .0708	.227 ± .027
Cardiovascular lesions				
Heart, Incidence	0	0	6	0
Heart, Severity	0	0	+ +	0
Kidney, Incidence	0	0	7	0
Kidney, Severity	0	0	+ +	0
Periarteritis, Incidence	0	0	5	0
Periarteritis, Severity	0	0	+ +	0

* Percentage of final body weight.
† Organ weights which differ significantly from those of the co-twin are indicated by the letter S.

any way with hypertension, since edematous rats might or might not be hypertensive and, conversely, hypertensive rats were as commonly free of edema as not.

Gross Findings at Autopsy.—Cardiac enlargement was obvious in all the hypertensive rats, and frequently the ventricles bore whitish patches. In such rats the kidney surfaces often bore small surface scars and glomerular hemorrhages which gave to them the so-called "flea-bitten" appearance. Rather large spherical or ovoid excrescences were commonly observed at intervals along the course of the branches of the superior mesenteric and splenic arteries (fig. 3). In most, although not in all, cases the thymus of the hypertensive partner was atrophic, and in some cases a remnant could not be identified with certainty; however, this also occurred occasionally in normotensive rats. The adrenals were larger, in general, in hypertensive animals than in their normotensive co-twins, although the adrenals of normotensive rats in parabiosis with hypertensive rats were larger than those found in normotensive rats in parabiosis together. The body and organ weights, together with their standard errors, and the incidence and the severity of cardiovascular lesions (graded on an arbitrary + to + + + scale) are given in the table.

Microscopic Observations.—Kidneys: While the kidneys of a normotensive rat, whether this was attached to a hypertensive or to a normotensive partner, were entirely normal structurally, those from a hypertensive animal invariably showed some degree of pathologic change. Hypertrophy and sclerosis of the media of the small arterioles were frequently encountered. In other areas sclerosis and

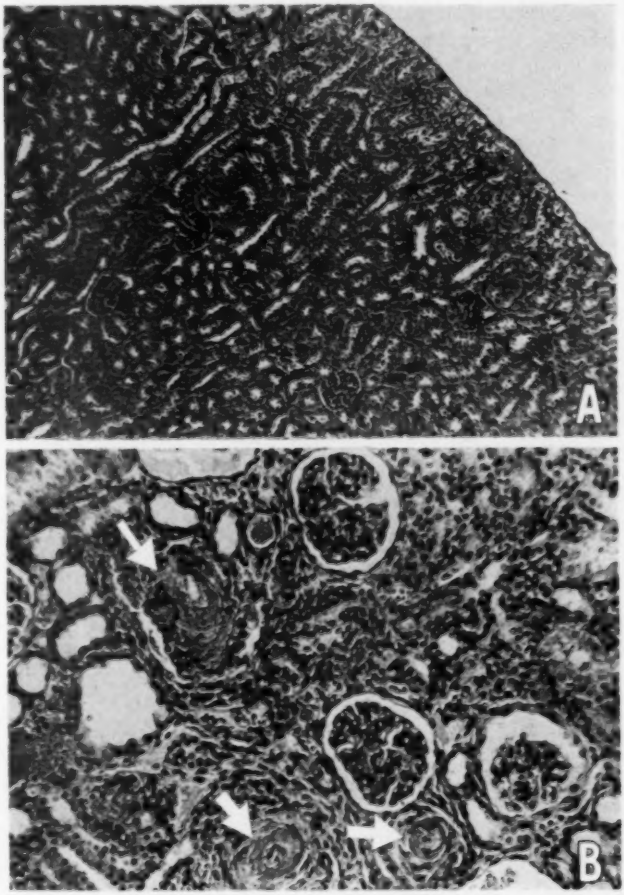

Fig. 4.—*A*, section of a kidney from the normotensive member of a pair of rats in parabiosis for 29 days. Normal histologic structure is evident. Hematoxylin and eosin; × 84.

B, section of a kidney from the hypertensive co-twin of the above rat. Sclerosed arterioles are indicated by arrows. Marked tubular dilatation is apparent. Hematoxylin and eosin; × 168.

hyalinization of the glomerular tuft capillaries, probably referable to sclerosis of afferent arterioles, constituted the prominent lesion. Occasionally, actual rupture of the glomerulus, with the formed elements of the blood being discharged into a distended Bowman's capsule, was observed. In such kidneys many of the tubules

were distended or plugged by casts of eosinophilic hyaline material (figs. 4 and 5). The lesions were primarily vascular and those of malignant nephrosclerosis.

Heart: In addition to the hypertrophy of individual myocardial fibers, hyalinization of cardiac arterioles, accompanied by a periarterial granulomatous reaction, was commonly encountered. In such areas histiocytes, leukocytes and

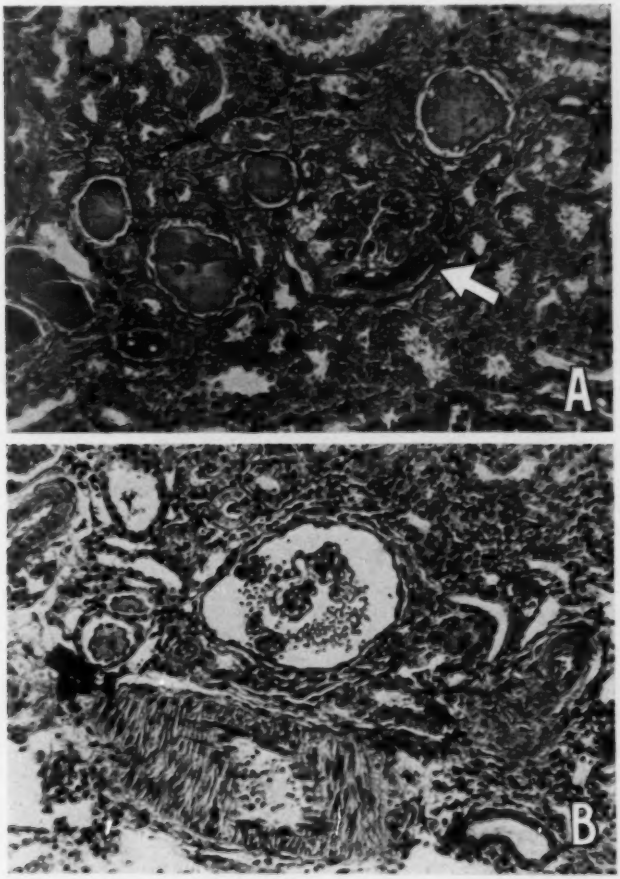

Fig. 5.—*A*, section of a kidney of the hypertensive member of a pair of rats in parabiosis for 36 days. Note the hyalinization of the capillary loops in the central glomerulus and the obliteration of the capsular space. The surrounding tubules contain hyaline casts. Hematoxylin and eosin; × 168.

B, section of a kidney of a hypertensive parabiotic rat, 29 days postoperatively. The enlarged and distended central glomerulus has ruptured, casting cells into the capsular space. Hematoxylin and eosin; × 168.

often polymorphonuclear giant cells were observed. These regions were strikingly similar to the so-called Aschoff nodules observed in the inflammatory manifestations of rheumatic heart disease. In some instances, areas containing degenerating and

necrotic myocardial fibers were encountered (figs. 6 and 7), the latter probably representing areas deprived of a normal blood supply by the vascular damage.

Pancreas and Mesentery: Examination of sections from the pancreas and branches of the superior mesenteric artery revealed arterial lesions in varying

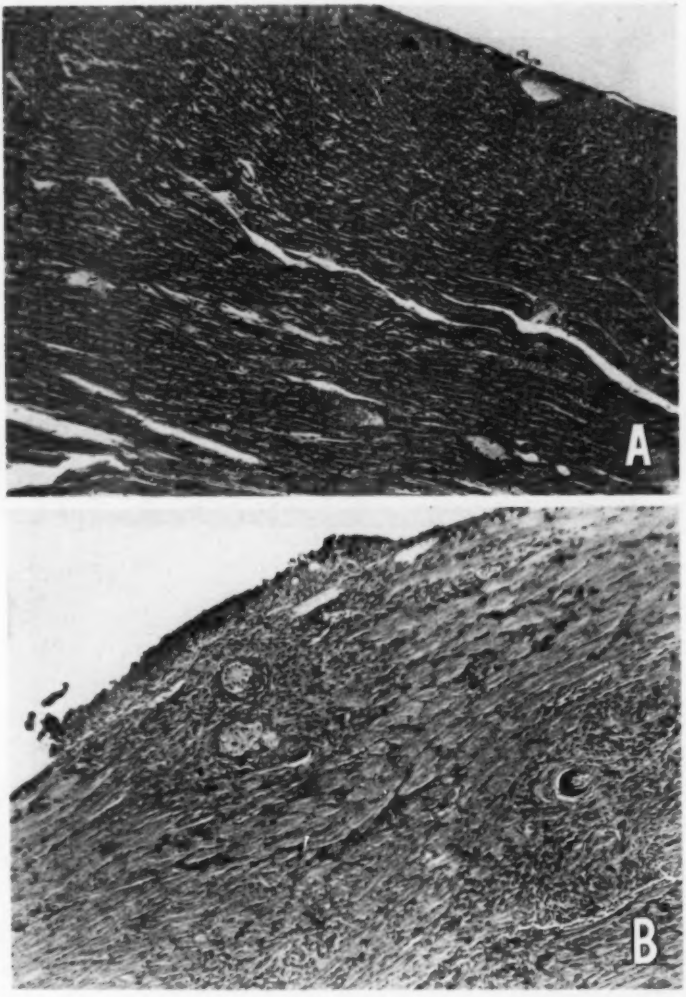

Fig. 6.—*A*, section of the normal ventricular myocardium of a normotensive rat in parabiosis for 29 days. Hematoxylin and eosin; × 84.

B, section of the left ventricle of the hypertensive co-twin of the above rat. Note hypertrophy of myocardial fibers. Several arterioles with necrotic, hyalinized walls may be seen in cross section, surrounded by a proliferative granulomatous reaction, similar to the "Aschoff nodule." Note the marked pericarditis at the surface of the heart. Hematoxylin and eosin: × 84.

degrees in most of the hypertensive rats. In most cases the arterial walls had lost their typical structure and had been transformed into a rather homogeneous hyaline material. In other areas, while the walls were not completely hyalinized, regions

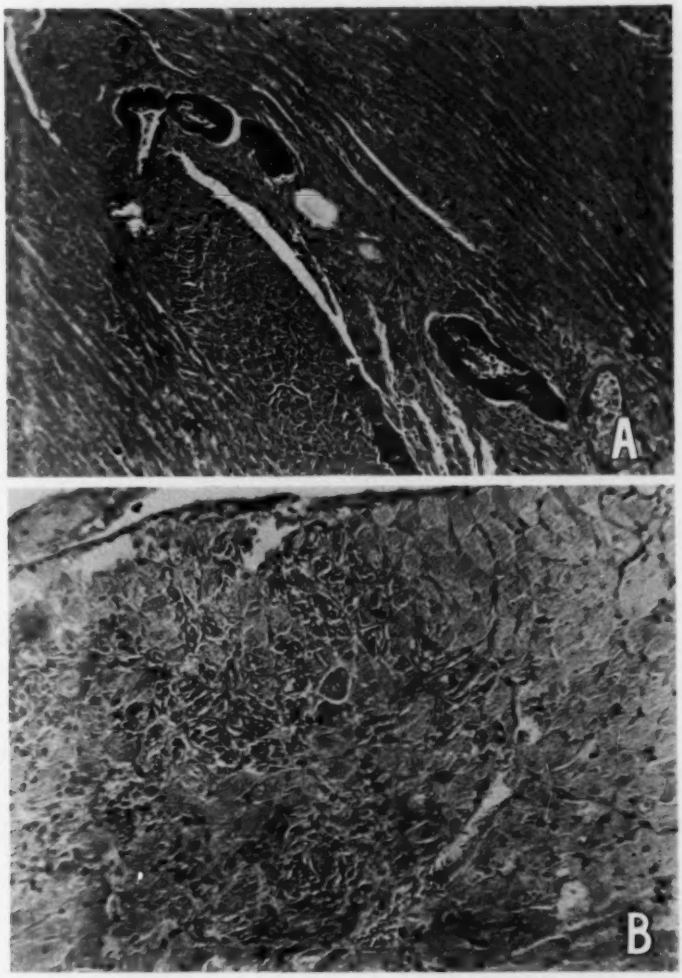

Fig. 7.—*A*, ventricular myocardium from a hypertensive rat in parabiosis for 36 days. Note the extreme hyaline necrosis of arteriolar walls and periarterial inflammatory reaction. Hematoxylin and eosin; × 84.

B, marked necrosis of cardiac muscle present in the left ventricle of the hypertensive member of a pair in parabiosis for 36 days. Hematoxylin and eosin; × 168.

of necrosis were apparent in them. Such vessels invariably were heavily invaded, in the adventitia and immediately adjacent tissue, by inflammatory cells, including

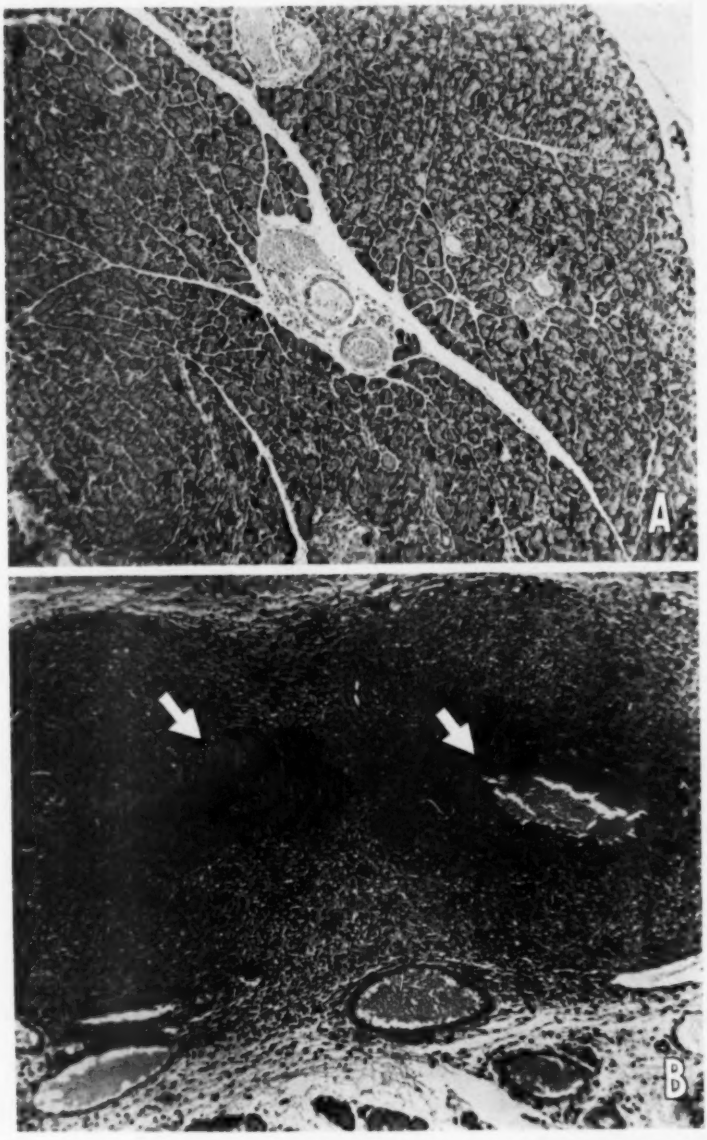

Fig. 8.—*A*, normal pancreas from the normotensive member of a pair of rats in parabiosis for 45 days.

B, periarteritis nodosa in the pancreas of the hypertensive co-twin of the above rat. Extreme hyaline necrosis of arterial walls (indicated by arrows), and inflammatory cell invasion of the adventitia.

lymphocytes and eosinophilic granulocytes. Occasionally the lumens of such vessels were occupied by thrombi. In brief, the lesions were essentially similar to those of periarteritis nodosa (fig. 8).

COMMENT

From this experiment it is apparent that malignant hypertensive vascular disease may be produced in the rat by the technique of parabiosis alone, without recourse to the various surgical interventions which have hitherto been employed to study hypertension in parabiotic rats. We have preliminary evidence at the present time that it is not essential to add sodium chloride to the diet, as reported here, to insure development of hypertension in parabiotic rats.[10]

The mechanism responsible for the induction of hypertension and associated cardiovascular lesions is not readily apparent, although three possible explanations suggest themselves. The response may be due to a stress reaction since overdosage with desoxycorticosterone acetate,[11] certain impure pituitary extracts [12] or stress in the form of exposure to cold [13] may produce similar lesions in the heart and the kidney, and it is known that parabiosis may elicit an "alarm reaction." [14] Against this view is the fact that while stress per se may produce nephrosclerosis there is, as Sayers [15] has emphasized, no experimental proof that it may elicit periarteritis nodosa. If a purely stressor reaction is involved, it is difficult to explain why only one animal is affected. Occasionally, one might expect, both partners would be affected, and yet apparently this is never the case. Conceivably, however, hypertension might result from the interaction between a specific damage confined to one of the partners and the nonspecific adrenal cortical hyperactivity characteristic of stress. It was particularly striking that the most advanced cardiovascular lesions could develop in as short a time as 21 days postoperatively, which is a shorter time than is necessary to produce similar lesions with any dosage of desoxycorticosterone acetate hitherto employed.

A second hypothesis which suggests itself is that the reaction is essentially a foreign serum allergic reaction. It may be that the serum of one partner contains substances toxic to the vascular system of the other. In this way periarteritis nodosa might result just as it does from treatment of rabbits with sterile horse serum.[16]

The third hypothesis is that the serum of one rat may contain specific substances which are toxic to the kidneys of the partner. In this way a renal hypertension similar in nature to that following the use of other renal cytotoxic serums [17] might result. Experiments designed to narrow the field of etiologic possibilities are in progress.

From the table it may be observed that the adrenals of both the hypertensive and the normotensive rat of a pair in which hypertension occurs in one are larger

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than those from normotensive rats in parabiosis together, although those from the hypertensive partner are larger than those from the co-twin. This is probably due to the fact that the adrenocorticotropic hormone (ACTH) is produced excessively by the pituitary of the hypertensive rat and crosses the anastomosis to enlarge the adrenals of the normotensive co-twin. It is known that ACTH readily crosses from one parabiotic animal to the other.[18] It is of interest to note that while adrenal cortical hypertrophy is apparent in both rats when one of them is hypertensive, only the hypertensive animal displays the cardiovascular lesions. There is no evidence at the present time to indicate with certainty whether the adrenal hypertrophy is the cause or the result of the hypertension, although the latter appears to be the more likely.

The conclusions derived from the results of this study are in harmony with those of Grollman and Rule,[2] who studied the effect of parabiosis on the induction and progress of hypertension induced by perinephritis in one of the pair. The histologically normal kidneys of a normotensive rat in parabiosis with a hypertensive co-twin do not appear to be able to prevent a malignant form of hypertension from developing in the latter, with the accompanying cardiovascular lesions. Nor does the hypertensive state in one partner produce any evidence of hypertension in the other. If a humoral agent is responsible for the hypertensive condition, either it does not cross the parabiotic capillary anastomosis or it is destroyed rapidly when it does. The latter hypothesis appears to be the more plausible.

SUMMARY

When rats are placed in parabiosis and maintained on a 1 per cent sodium chloride drinking solution, malignant hypertensive vascular disease develops in one of the partners in about 40 per cent of the cases. This is usually preceded, or accompanied at the first evidence of hypertension, by marked hypotension in the attached co-twin.

The hypertensive state is accompanied by renal and cardiac hypertrophy, nephrosclerosis, periarteritis nodosa and rheumatic myocardial lesions.

The adrenal cortices of both rats when one is hypertensive are much larger than those from normotensive rats in parabiosis. The adrenals of the hypertensive partner, however, tend to be larger than those of the normotensive co-twin.

The pathogenesis and the significance of the syndrome of parabiotic hypertension are discussed.

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CYTOLOGY OF HYPERPLASTIC PANCREATIC ISLETS IN ADDISON'S DISEASE

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MORPHOLOGIC changes in the islets of Langerhans in patients with Addison's disease should be anticipated in view of the serious alterations which occur in carbohydrate metabolism in that disease. This is especially true in consideration of the fully justified conclusions of Cramer [1] that "the islets of Langerhans constitute a very plastic tissue which undergoes considerable changes in a variety of physiological conditions, even to the extent of the formation of new islets on the one hand, and the disappearance of existing islets on the other." In the 18 cases of Addison's disease forming the basis of this report, striking qualitative and quantitative changes have been found in pancreatic islets.

Since 1908 evidence has been accumulating that adrenal cortical insufficiency is accompanied with abnormalities in carbohydrate metabolism.[2] Thorn, Koepf, Lewis and Olsen [3] compiled a complete list of the functional derangements of carbohydrate metabolism in patients with Addison's disease. These included a low fasting blood glucose level, striking hypoglycemia, decreased threshold at which symptoms of hypoglycemia appear, flat type of oral glucose tolerance curve, absence of "rebound" in blood sugar curve following intravenous administration of dextrose, and a high standard respiratory quotient which increased following administration of dextrose. These findings were in agreement with those in animals following adrenalectomy.[4] Kepler and Willson [5] reemphasized the importance of hypoglycemia and depletion of liver glycogen as claimed earlier by Britton and Silvette.[6] Long and Lukens [7] demonstrated a mutual antagonism between the adrenal cortical hormones and insulin in a depancreatized, adrenalectomized dog. The diabetic state of the previ-

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ously depancreatized dog was greatly improved by adrenalectomy. There has been a very intensive research into these functional abnormalities, which has been summarized by Jensen [8] and Noble.[9]

The reports of cases of uncomplicated Addison's disease do not include descriptions of changes in the islets of Langerhans.[10] However, Susman [11] included one case of Addison's disease in a miscellaneous series in which pancreatic islets were studied quantitatively. He found a definite increase in islet tissue, but no detailed study of this case was presented.

Twenty cases of concomitant Addison's disease and diabetes mellitus have been reported. A striking feature of most of these cases was the marked improvement in the preexisting diabetes mellitus, as shown by a profound reduction of the amount of exogenous insulin required.[12] This was further evidence of an antagonism between the adrenal cortical hormones and insulin. In two of these cases [13] the islets of Langerhans were examined thoroughly by Dr. Gomori. The changes reported were characteristic of most cases of diabetes mellitus [14] and not like those of uncomplicated Addison's disease.

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8. Jensen, H.: The Internal Secretion of the Pancreas, in Pincus, G., and Thimann, K. V.: The Hormones: Physiology, Chemistry and Applications, New York, Academic Press, Inc., 1948, vol. 1, p. 301.

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10. Barker, N. W.: The Pathologic Anatomy in 28 Cases of Addison's Disease, Arch. Path. **8**:432-450 (Sept.) 1929. Guttman, P. H.: Addison's Disease: A Statistical Analysis of 566 Cases and a Study of the Pathology, ibid. **10**:742-785 (Nov.); 895-935 (Dec.) 1930. Susman, W.: Atrophy of Adrenals Associated with Addison's Disease, J. Path. & Bact. **33**: 749-760 (July) 1930. Duff, G. L., and Bernstein, C.: Five Cases of Addison's Disease with So-Called Atrophy of Adrenal Cortex, Bull. Johns Hopkins Hosp. **52**:67-85 (Jan.) 1933. Duffin, J. D.: Cortical Necrosis of the Adrenal Glands Associated with Addison's Disease: Report of 8 Cases, Arch. Path. **35**:649-666 (May) 1943. Helve, O.: Study of Metabolism in Addison's Disease: On Carbohydrate and Phosphorus Metabolism, Acta med. scandinav. **127**:543-564, 1947. Moehlig, R. C.: Addison's Disease Followed for 9 Years: Case Report with Autopsy, J. Clin. Endocrinol. **7**:134-151 (Feb.) 1947. Friedman, N. B.: Pathology of Adrenal Gland in Addison's Disease with Special Reference to Adrenocortical Contraction, Endocrinology **42**:181-200 (March) 1948. Heller, E. L., and Camarata, S. J.: Addison's Disease from Amyloidosis of Adrenal Glands, Arch. Path. **49**:601-604 (May) 1950.

11. Susman, W.: Quantitative Variations of Pancreatic Islet Tissue in Mixed Series of Cases, J. Clin. Endocrinol. **2**:97-106 (Feb.) 1942.

12. (*a*) Rhind, E. G. G., and Wilson, A.: Diabetes Mellitus in Addison's Disease, Lancet **2**:37-38 (July 12) 1941. (*b*) Thorn, G. W., and Clinton, M., Jr.: Metabolic Changes in Patient with Addison's Disease Following Onset of Diabetes Mellitus, J. Clin. Endocrinol. **3**:335-344 (June) 1943. (*c*) Devitt, J. S., and Murphy, F. D.: Diabetes Mellitus Complicated by Addison's Disease: Case Report with Review of Literature, Am. J. Digest. Dis. **14**:164-165 (May) 1947. (*d*) Bernstein, D. E.: Diabetes Mellitus Followed by Addison's Disease and Hypothyroidism, Simulating Panhypopituitarism, J. Clin. Endocrinol. **8**:687-693 (Aug.) 1948. (*e*) Crampton, J. H.; Scudder, S. T., and Davis, C. D.: Carbohydrate Metabolism in the Combination of Diabetes Mellitus and Addison's Disease, as Illustrated by a Case, ibid. **9**:245-254 (March) 1949. (*f*) Knowlton, A. I., and Kritzler, R. A.: The Development of Diabetes Mellitus in Addison's Disease: Case Report with Autopsy, ibid. **9**:36-47 (Jan.) 1949.

13. Bernstein.[12d] Knowlton and Kritzler.[12f]

14. (*a*) Gomori, G.: Observations with Differential Stains on Human Islets of Langerhans, Am. J. Path. **17**:395-406 (May) 1941; (*b*) Histology of Normal and Diseased Pancreas, Bull. New York Acad. Med. **21**:99-109 (Feb.) 1945. (*c*) Bell, E. T.: The Beta Granules in the Islets of Langerhans in Diabetes Mellitus, Am. J. Path. **22**:631 (May) 1946.

Alterations in pancreatic islets were not mentioned in descriptions of adrenal cortical insufficiency in experimental animals in which certain morphologic changes were emphasized.[15] The prevailing hypothesis was that the anti-insulin effect of the adrenal cortex appeared to be a specific peripheral action between the hormones of adrenal cortex (with those of the hypophysis) and insulin.[16] A central effect of the adrenal cortical hormones directly on the islets of Langerhans was not considered by most workers.

MATERIAL

Eighteen cases of Addison's disease formed the basis for this study. The same cases were utilized by O'Donnell[17] for his investigation of the pathogenesis of Addison's disease. In this series the destruction of the adrenal cortex was caused by caseating tuberculous adrenalitis in eight cases, so-called primary atrophy in five, generalized amyloidosis in four, and histoplasmosis in one case. The range of the patients' ages was from 27 months to 70 years. Eight patients were males. Five of the 18 patients received some hormonal substitution therapy, but by present day standards this therapy was inadequate, especially in reference to any influence on carbohydrate metabolism. Seven of the 18 patients were emaciated; eight gave evidence of good nutrition; the remaining three were judged to have only fair nutrition.

METHODS AND RESULTS

Blocks were taken from four to six different areas of each pancreas to eliminate possible errors in sampling. The tissue had been fixed in formaldehyde solution U. S. P. and stored in alcohol or formaldehyde solution U. S. P. The blocks were refixed in Bouin's solution. The staining procedures used were the routine hematoxylin and eosin stain, Gomori's chromium hematoxylin and phloxine stain,[14a] the Mallory-Heidenhain azocarmine stain, and Mallory's phosphotungstic acid-hematoxylin.

Hyperplasia of Islet Tissue.—The striking features of the islets of Langerhans in each case were the evidences of marked and active hyperplasia as shown by increased size (fig. 1), by increased numbers (fig. 2) and by active neogenesis of islets (figs. 3 and 4). The islet tissue constituted a much greater percentage of the total pancreas in patients with Addison's disease than in those dying of other diseases in which the pancreas was considered normal.

Otani[18] concluded that the new formation of pancreatic islets in the adult human probably occurred by transformation and hyperplasia of acinar, centroacinar and duct cells and possibly by development of undifferentiated pancreatic tissue. In the cases of Addison's disease numerous examples of proliferating islet tissue were found which suggested the above possibilities. The marked increase in small ducts and the transition of duct epithelium to islet cells with specific granules were particularly striking (fig. 4). The streaming of islet cells from the periphery of mature islets was present in some cases, even to the point of fusion of two or more islets. In some cases a complete ring of islet tissue encircled ducts. Immature proliferating islet cells were spread diffusely among acinar structures to a pronounced degree (fig. 3). Many times these elements were so intermingled that I was unable to delineate

15. MacMahon, H. E., and Zwemer, R. L.: Pathologic Histology of Adrenalectomized Cats, Am. J. Path. **5**:491-498 (Sept.) 1929. Rogoff, J. M.: Experimental Pathology and Physiology of the Adrenal Cortex: Production of Addison's Disease in Laboratory Animals, Arch. Path. **38**:392-409 (Dec.) 1944.

16. Kendall, E. C.: The Adrenal Cortex, Arch. Path. **32**:474-501 (Sept.) 1941.

17. O'Donnell, W. M.: Changing Pathogenesis of Addison's Disease, with Special Reference to Amyloidosis, Arch. Int. Med. **86**:266-279 (Aug.) 1950.

18. Otani, S.: Studies on the Islands of Langerhans in Human Pancreas: I. The Relation of the Islands to the Surrounding Structures, Am. J. Path. **3**:1-11 (Jan.) 1927; II. Significance of Variations in Structure, ibid. **3**:123-134 (March) 1927.

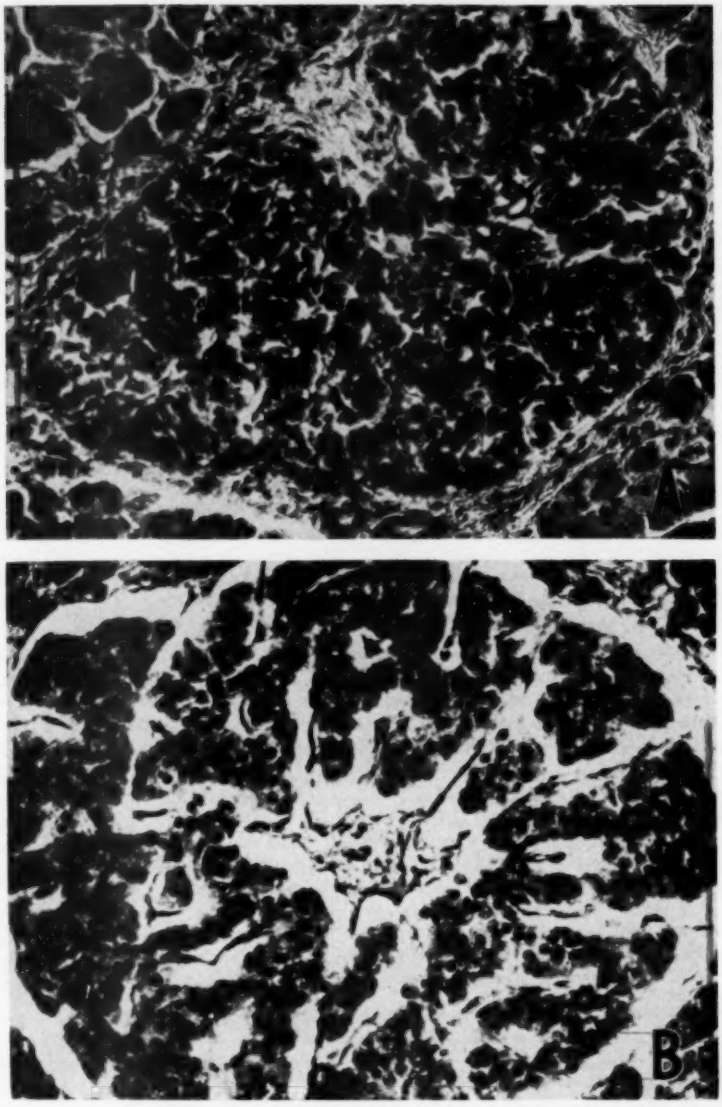

Fig. 1.—These and the succeeding photographs have been prepared from sections of pancreases of patients with Addison's disease. *A* shows a young hyperplastic pancreatic islet consisting of small oblong or spindle-shaped cells in a compact arrangement. The darker-staining cells are beta cells with abundant granules. The remainder of the cells, making up the majority, resemble alpha cells. Gomori's chromium hematoxylin and phloxine stain; × 300.

B, a more mature hyperplastic islet than that shown in *A*, consisting of large polygonal cells, the majority of which are alpha cells. Gomori's chromium hematoxylin and phloxine stain; × 300.

them with any degree of certainty. Mitotic division of preexisting islet cells did not account for much of the increase in islet tissue. This is in agreement with the findings of Otani and others. An occasional division figure was seen in 10 of the 18 cases of Addison's disease. It should be emphasized that destructive lesions were not found in the pancreas to explain the striking degree of hyperplasia of islets.

The evaluation of changes in volume of islet tissue always has been a serious problem. Volumetric determinations based on fixed human pancreas have been unsatisfactory. Many authors have expressed volumetric measurements of islet tissue in terms of percentage of total volume of pancreatic tissue. Such an expression of one variable in relation to another independent variable must be considered of but little value. In 12 of the 18 cases in this series the total weight of the pancreas was considered normal, while in six cases the weight was below normal. Microscopically, the acini were considered normal in size in three cases, slightly decreased in size in two cases, moderately atrophic in seven cases and markedly atrophic in six cases. If the amount of islet tissue in the latter cases had been normal, the practice of

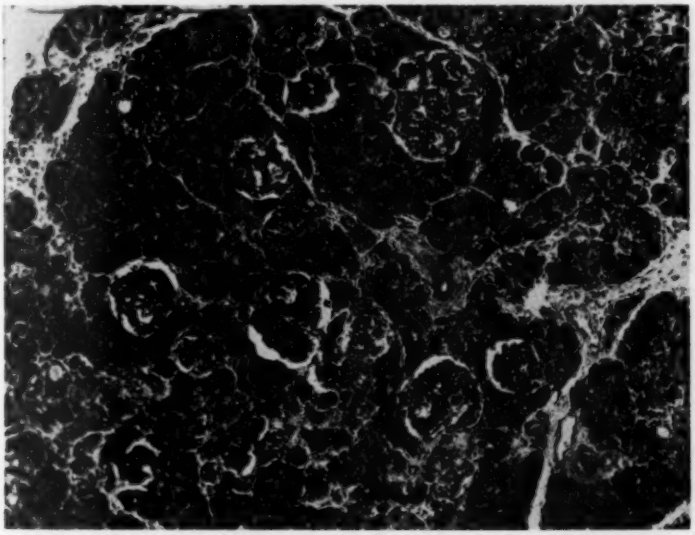

Fig. 2.—Low power view of the pancreas in a case of Addison's disease, demonstrating the increase in number of large mature islets. Gomori's chromium hematoxylin and phloxine stain; × 120.

expressing the volume as a percentage of the total pancreatic tissue, as followed by the above authors, would have resulted in the false concept of an exaggerated increase in volume of islet tissue. However, according to his excellent monographic work on quantitative determination of islet volume, Tejning [19] found mathematically that if one encountered unusually large islets or more than the usual number of large islets, an increased volume of islet tissue was assured. I reached similar conclusions after measuring the mean diameters of many thousands of islets.

Among the cases of Addison's disease many mature islets were considerably larger than normal (fig. 1). This was reflected in a total increase in size of islets. As a rough determi-

19. Tejning, S.: Dietary Factors and Quantitative Morphology of Islets of Langerhans: Experimental Study of Influence of Caloric Composition of Diet on Islets of Langerhans and Analysis of Relation Between Volume of Islet Tissue and Number of Islets in Rat, Acta med. scandinav., supp. 198, pp. 1-154, 1947.

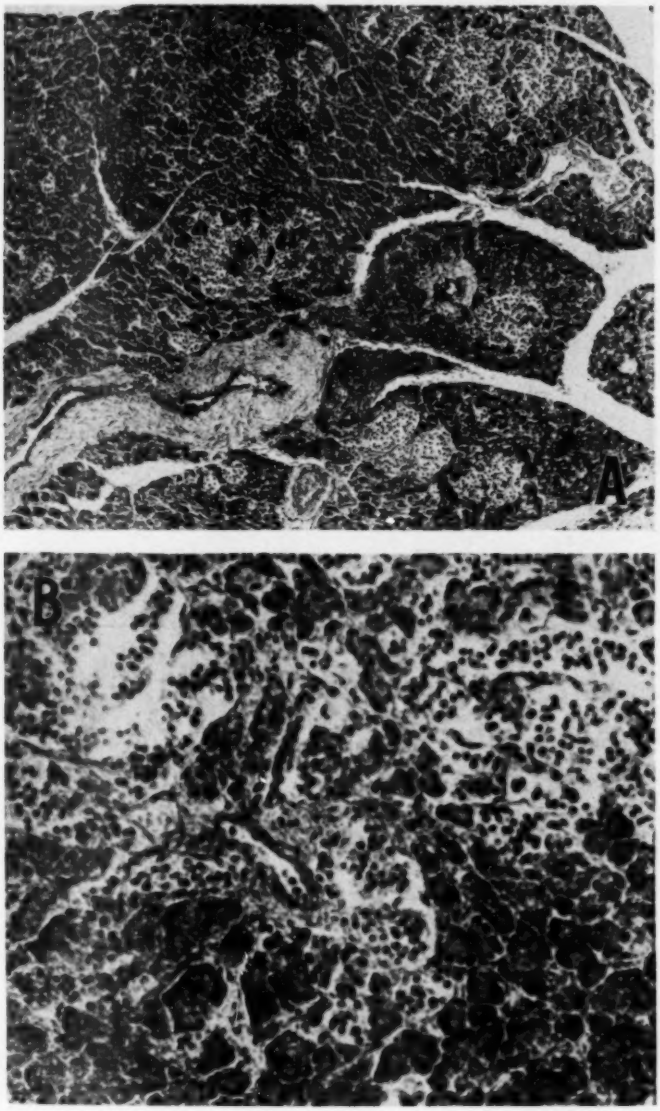

Fig. 3.—*A*, pancreas in a case of Addison's disease showing abundant islet tissue, much of which is seen to stream diffusely among the acinar structures. Gomori's chromium hematoxylin and phloxine stain; × 55.

B, higher power view of the section used for *A*, showing the streaming of immature islet tissue from a duct. Gomori's chromium hematoxylin and phloxine stain; × 250.

nation of this feature, 35 islets in a random consecutive series from each case of Addison's disease and from each of 10 normal control cases[20] were measured in their longest diameters with a calibrated ocular micrometer. In the normal controls the average of the long diameters in the individual case varied from 128.8 microns to 156.5 microns. The over-all average for all the controls was 142.4 microns. In the cases of Addison's disease the average of the long diameters in the individual case varied from 174.2 microns to 260.0 microns, with an over-all average of 201.5 microns. Accordingly, the long diameters of the islets in cases of Addison's disease in this series were increased to 141.5 per cent of those of normal islets.

Due allowances being made for the varying degrees of acinar atrophy in Addison's disease, the total number of islets was increased very strikingly over that of the normal pancreas (fig. 2). The streaming of proliferating islet cells among acini (fig. 3) and the coalescence of many islets left no doubt about an increase in number of islets, but these features made accurate counts more difficult. However, as a rough indication of the increase, actual counts of only the mature islets (those in which beta granules were identified) in areas 6 mm. in diameter were done. In the

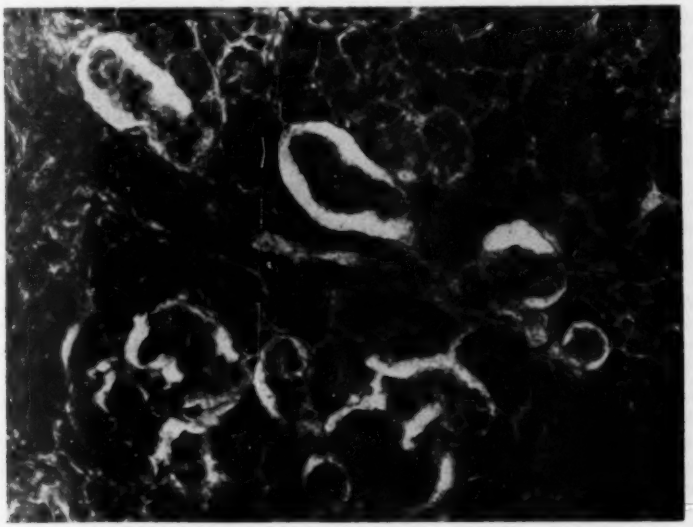

Fig. 4.—Small masses of proliferating immature islet cells surrounding a small duct, from which they appear to have originated. In a few of the cells in the lower left field alpha and beta granules already have developed. Gomori's chromium hematoxylin and phloxine stain; × 300.

normal controls the average number of islets varied from 12 to 28, with an over-all average of 19. In the cases of Addison's disease the number of mature islets varied from 52 to 181, with an over-all average of 92.5. This represents approximately a fivefold increase.

The example of Addison's disease in a 27 month old infant was eliminated from the last two series of determinations because of the possibility of normal neogenesis of islet tissue at that age and because of the greater normal ratio of islet to acinar tissue in infancy. However, in five of the remaining cases of Addison's disease the combination of very active genesis of islets and pronounced atrophy of acinar tissue resulted in a picture strikingly like that of fetal or infantile neogenesis of islets.

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20. The normal controls were carefully selected from a large series of 500 consecutive necropsies in which there had been a routine analysis of islet tissue, both quantitative and qualitative, including the use of the specific granule stains mentioned earlier.

It should be emphasized that the measurements and the counts of islets were subject to considerable chance for error. In fact, they were intended only as a crude indication of the general trend. In such material it is impossible to make an accurate determination of total islet volume by any method now available. This fact, combined with the obvious increase in volume of islet tissue, rendered more exhaustive quantitative determination pointless. However, as crude as the determinations were, the magnitude of the increase in size and number of the mature islets alone and the lack of overlapping between the two groups of cases substantiated the validity of the observations. If there had not been a pronounced difference, the results could not have been accepted as significant. The increase in islet tissue was far greater than could be expected to result from errors inherent in the method.

Types of Islet Cells Involved in the Hyperplasia.—Gomori's procedure has proved to be the most useful and efficient of staining methods for the islets of Langerhans of man,[21] and especially with material of this type. It clearly distinguished beta cells from alpha and delta cells on the basis of cytoplasmic granules of contrasting blue and red. The other two special stains differentiated alpha and delta cells, at present a relatively unimportant determination in view of their constant ratio and the small percentage of delta cells.[22]

The proportion of beta to alpha and delta cells was determined by counting 1,000 random cells from each of the 18 cases of Addison's disease and from each of the control cases. The beta cells of the controls were in the normal range of 75 to 90 per cent, with an average of 82 per cent. The alpha and delta cells constituted the remaining 18 per cent of islet cells. There was an even wider variation in the islets from cases of Addison's disease. Here the percentage of beta cells ranged from 36 to 76 per cent, with an average of 52 per cent (a difference of −30 per cent, and a decrease of 36.6 per cent as compared with the control material). Conversely, the alpha and delta cells ranged from 24 to 64 per cent, with an average of 48 per cent. Three cases of Addison's disease showed a beta cell count of 70 to 76 per cent (lower limits of normal). In the remaining 15 cases there was a definite reduction in the ratio of beta to alpha and delta cells. In eight cases the percentage of beta cells was 50 or less (considered a critical level for most cases of diabetes mellitus [14a, b]). However, the total increase in islet cells was so great that it more than offset the decrease in percentage of beta cells. The beta cells obviously were increased in number, with a proportionately greater increase in cells of the alpha-delta series.

State of Granulation of Islet Cells.—In Addison's disease the beta cells possessed unusually abundant beta granules. In five cases there were focal areas of partial degranulation, but the total granulation was greater than normal in all the cases. This probably was indicative of a storage phenomenon, possibly due to a reduced peripheral need for insulin in Addison's disease. As far as could be determined there were no changes in granulation in alpha cells.

CONCLUSIONS

There can be no doubt that the terminal stage of Addison's disease is characterized by a profound hyperplasia of islets of Langerhans, as shown by increased size and number of islets and by very active neogenesis of islets or islet cells. This is a previously undescribed feature of Addison's disease. It would seem that in some way adrenal cortical insufficiency plays an important role in inducing islet hyperplasia, since the increase in well granulated beta cells is contrary to the changes anticipated in view of the reported alterations of carbohydrate metabolism.

21. (*a*) Gomori, G.: A Differential Stain for Cell Types in the Pancreatic Islets, Am. J. Path. **15**:497-499 (July) 1939. (*b*) Barron, S. S.: Significance of Beta Granules in Islets of Langerhans of Pancreas, Arch. Path. **46**:159-163 (Aug.) 1948. (*c*) Conn, J. W., and Hinerman, D. L.: Effects of Alloxan upon Function and Structure of Normal and Neoplastic Pancreatic Islet Cells in Man, Am. J. Path. **24**:429-449 (March) 1948. (*d*) Gomori.[14a,b] (*e*) Bell.[14c]

22. Gomori.[14a,b] Gomori.[21a]

Preliminary studies of pancreatic islets [23] of patients receiving a preparation of adrenocorticotropic hormone (ACTH) and cortisone suggest that there may be some inhibitory factor operating directly on the islets of Langerhans in the presence of adrenal cortical hormones. Such possibilities are a potent source of speculation, but such speculation is beyond the scope of this paper.

The increase in all types of islet cells in Addison's disease, with a significant reduction in the ratio of beta to alpha and delta cells in most of the cases, indicates the relative lack of importance of that ratio. The all-important feature is the total number of healthy functioning beta cells in relation to metabolic needs. It should be emphasized that all types of islet cells are increased, but the increase is greater in alpha and delta cells than in beta cells.

The unusual abundance of beta granules possibly is due to storage as a result of a reduction in the peripheral demand for insulin in Addison's disease. It does not mean necessarily that there is increased production and liberation of insulin, even though a close relationship exists between insulin and beta granules.[24]

The above findings serve to emphasize Cramer's conclusion that the islets of Langerhans are very labile organs which readily undergo morphologic changes under a variety of conditions, one of which may be the loss of the adrenal cortex.

SUMMARY

Hyperplasia of islets of Langerhans, previously undescribed, was a striking feature of 18 cases of Addison's disease.

This hyperplasia was characterized by marked increase in both number and size of islets and by very active neogenesis of islet cells.

An increase in all types of islet cells was present, but alpha and delta cells were increased to a somewhat greater degree than beta cells.

The beta cells possessed unusually abundant beta granules, which are closely related to insulin.

Results of this study emphasize Cramer's statement that the islets of Langerhans are very labile organs which readily undergo morphologic changes under a variety of conditions. The presence or absence of functioning adrenal cortex appears to be one of these varying conditions.

23. Hinerman, D. L.: Unpublished data.

24. Best, C. H.; Campbell, J., and Haist, R. E.: Effect of Anterior Pituitary Extracts on Insulin Content of Pancreas, J. Physiol. **97**:200-206 (Dec. 14) 1939. Ham, A. W., and Haist, R. E.: Histological Study of Trophic Effects of Diabetogenic Anterior Pituitary Extracts and Their Relation to the Pathogenesis of Diabetes, Am. J. Path. **17**:787-812 (Nov.) 1941. Best, C. H.; Campbell, J.; Haist, R. E., and Ham, A. W.: Effect of Insulin and Anterior Pituitary Extract on Insulin Content of Pancreas and Histology of Islets, J. Physiol. **101**: 17-26 (June 2) 1942. Barron.[21b]

Case Reports

CONGENITAL FIBROSARCOMA

Report of a Case in a Newborn Infant

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AND
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WINSTON-SALEM, N. C.

IT IS EXTREMELY rare for any type of cancer, particularly fibrosarcoma, to be present in a newborn infant. Wells,[1] in a complete review of the literature published in 1940, found 250 cases in which possible cancers were present at birth. Of these, only 66 could be established beyond doubt as cancerous growths. Approximately one half of these 66 tumors were sarcomas, and nine of this group were fibrosarcomas. Of the nine infants with fibrosarcoma, two were reported as "recovered," while the ultimate outcome in two others was unknown. All the remaining five patients died within the first seven months of life.

The following report of a case of fibrosarcoma in a newborn infant is the tenth to be recorded in the literature.

CLINICAL HISTORY

B. S., a 24 hour old boy, was transferred to the North Carolina Baptist Hospital on March 1, 1948 because of multiple skin lesions noted at birth. He had received six injections of penicillin before transfer, because a tentative diagnosis of congenital syphilis had been made.

The referring hospital reported that Kahn and Mazzini tests of the mother's blood were positive at the time of the infant's birth, but that Kahn and Mazzini tests of blood from the cord were negative. No abnormalities of the placenta were noted.

Family History.—The mother's blood gave a positive reaction for syphilis two years prior to the baby's birth, and she received 6 or 7 million units of penicillin and 15 to 20 injections of oxophenarsine hydrochloride (mapharsen®) and bismuth. One year later her blood test was still positive, and she was given an additional 10 million units of penicillin and six injections of oxophenarsine hydrochloride and bismuth. She received no antisyphilitic therapy during pregnancy, and the Kahn test of her blood was negative at the end of the third month of pregnancy. She was apparently well except for some edema of the ankles in the last trimester of pregnancy. The family physician found no evidence of cancer in the mother. The family history was entirely negative for cancer, but the history was considered unreliable.

Physical Examination.—When admitted, the infant had a rectal temperature of 100.2 F. and weighed 6 pounds, 7 ounces (2,920 Gm.). General examination revealed a vigorous, well developed and well nourished boy with a hoarse cry and multiple lesions of the skin. These were hard, elevated, white nodular lesions involving the skin and subcutaneous tissue and measuring from 2 mm. to 3 cm. in diameter (fig. 1). The larger lesions were eroded. Most of

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1. Wells, H. G.: Occurrence and Significance of Congenital Malignant Neoplasms, Arch. Path. **30**:535-601 (Aug.) 1940.

the lesions were scattered over the trunk and posterior surface of the body, but there were a few small nodules on the scalp, the lip and the palate. Three bullae were present on the soles of the feet. There were no rhagades or nasal or anal lesions. The liver was palpable 2 cm. below the right costal margin and was smooth and firm. The spleen was barely palpable. The remainder of the physical examination gave negative results.

Laboratory Studies.—The hemoglobin content was 15 Gm., the red blood cell count 5,600,000 per cubic millimeter and the white blood cell count 22,000 per cubic millimeter. A differential count showed 70 polymorphonuclear granulocytes, 2 eosinophils, 25 lymphocytes and 1 normoblast. Thrombocytes (platelets) were normal in number and appearance. The urine was negative

Fig. 1.—Multiple skin nodules as they appeared on the second day of life. The nodules were elevated and had a rubbery consistency. Some showed ulceration of the surface with crusting.

for spirochetes. The stools were soft and black, and the guaiac test was positive. Serologic tests for syphilis showed the following results: March 2, Wassermann test doubtful, Kahn test negative; March 3, Kahn test negative; March 5, Wassermann test positive; March 17, Kahn test negative; March 19, Kahn test negative.

Roentgenograms of the chest, wrists, forearms, knees and femurs revealed no abnormality.

Progress.—On March 2, 1948, the infant was seen by a dermatologic consultant. He was impressed by the hard, rubbery character of the skin lesions and felt that they represented a "new growth with multiple metastases, the primary lesion likely being an internal one." A biopsy of one of the nodules showed fibrosarcoma.

The infant was given supportive care only, and he became progressively worse. His skin lesions increased in size and number, and some of the new lesions were entirely subcutaneous. One such lesion in the left thigh measured 4 by 6 cm. He became much more hoarse and was occasionally cyanotic. He lost 18.5 ounces (524.5 Gm.) in weight. Because of the hopeless prognosis, he was discharged on March 17 at the family's request. He died on March 22, 1948, on the twenty-second day of life.

NECROPSY

The body was that of an emaciated, poorly developed and poorly nourished white male infant, who weighed 2,100 Gm. and measured approximately 50 cm. in length. Scattered over the entire body, including the scalp, were numerous raised, rubbery, firm areas varying from 3 mm. to 4 cm. in diameter. The majority of these nodules measured from 1 to 1.5 cm. in diameter. A few nodules on the trunk of the body were flattened and presented a crusted surface. The latter lesions were raised and were surrounded by a rather firm, somewhat indurated edge. One small lesion, measuring 4 mm. in dameter, was situated on the left lower eyelid. On the right eyelid there was a similar lesion, measuring approximately 3 mm. in diameter. On the under surface of the right side of the tongue there was another elevated nodule, measuring 1 cm. in diameter.

The extremities presented no lesions other than the superficial ones described and a large swelling over the anterior surface of the left femur. This mass was rubbery in consistency, and on palpation appeared to be attached to the bone. It measured approximately 4.5 cm. in length and 2.5 cm. in width.

In the peritoneal cavity numerous small raised white nodules of a hard, rubbery consistency were noted over the entire surface of the bowel. The largest of these nodules measured 5 mm. in diameter. The liver weighed 75 Gm. and revealed nodules both on the surface and within the parenchyma. The pancreas weighed 4.5 Gm., and the surface presented numerous nodules, which were grayish white. The kidneys were lobulated but otherwise showed no gross changes.

In the pleural cavity were nodules of the same type, the largest of which was 7 mm. in diameter. These nodules appeared to be situated beneath the parietal pleura. The larynx revealed many firm white nodules attached to the external surface of the muscles in this region. The largest of these measured 9 mm. in diameter. The largest single group of nodules observed was found on the surface of the thyroid cartilage. Along the edge of the muscle attached to the trachea there were many similar nodules. A few scattered white nodules were seen on the diaphragmatic surface of both lungs. Section of the lungs revealed white nodules measuring as much as 5 mm. in diameter within the parenchyma.

The pericardial cavity revealed no gross abnormalities. The anterior surface of the heart contained the same nodules. On section the wall of the right ventricle was seen to be infiltrated with numerous grayish white nodules.

MICROSCOPIC OBSERVATIONS

The nodules in all sections of the body showed essentially the same microscopic picture (fig. 2). The cells were fusiform and had a rounded, slightly reticular nucleus with somewhat elongated cell processes. There was a slight tendency to fasciculation in some of the tumor nodules. Sections within the same tumor were somewhat variable; however, the over-all picture was uniform. At the periphery of some of the nodules there was a tendency toward invasion of the surrounding

tissues. Masson's trichrome stain showed the cells to be irregular in size, shape and staining properties and to have elongated and hyperchromatic nuclei.

The lungs were profusely infiltrated by polymorphonuclear neutrophilic granulocytes; this infiltration was particularly prominent in the interstitial spaces. Many of the bronchioles contained an inflammatory exudate.

ETIOLOGIC CONSIDERATIONS

The fact that fibrosarcoma was present at birth suggests some possible prenatal influence. The possibility of a hereditary factor was considered, but no family history of cancer could be obtained. Schnorbusch and Kujath [2] have reported that they could find no unusual mortality from cancer in the relatives of cancer patients under 35 years of age.

Some significance may be attached to the fact that the mother had been treated for syphilis. The Kahn test was made on her blood repeatedly, with negative

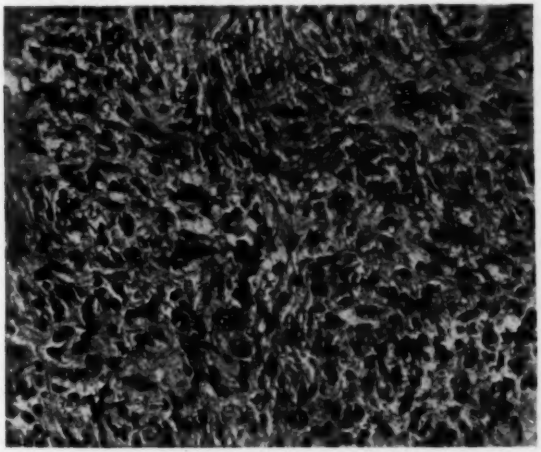

Fig. 2.—High power photomicrograph of a tumor nodule in the lung; × 145. The cells are irregular, hyperchromatic and fusiform, and show loss of polarity. An occasional mitotic figure is seen.

results until late in her pregnancy. Several examinations of the infant's blood resulted in only one positive serologic reaction for syphilis. Certainly the infant had none of the clinical findings typical of congenital syphilis, and microscopic examinations post mortem showed no evidence of syphilis. What part, if any, the mother's infection may have played in the production of the fibrosarcoma, we cannot say. It is, of course, known that fibrous nodules can be formed in syphilis and whether or not they may be as widespread as in the present case, we cannot say.[3] The microscopic sections of the tumors show definite fibrous nodules, many of which have undergone fibrosarcomatous changes. The sections could not in any way, we believe, be interpreted as representing a gumma.

2. Schnorbusch, M. T., and Kujath, B.: Untersuchungen in den Familien jugendlicher Krebskranker, Ztschr. menschl. Vererb.- u. Konstitutionslehre **21**:676, 1938.

3. Stout, A. P.: Personal communication to the authors.

RELATION OF CANCERS IN NEWBORN INFANTS TO THOSE IN ADULTS

It would appear that there is a fundamental difference between the cancers which occur in adults and those which are present in the newborn. Although there are no striking microscopic differences between the sarcomas seen in adults and those seen in newborn infants, it is probable that the tumors might be quite different with regard to causes. Faerber [4] stated that "it is irrational to work on the hypothesis that cancer afflicts cells and tissues of all ages through the same mechanism when the cancer of infancy may constitute a separate category by virtue of the peculiar metabolic properties of the tissues of their origin."

A study of congenital neoplasms reveal that sarcoma is probably the only cancer occurring in adult life which is likely to be present at birth. Wells,[1] in a complete review of the literature, could not find a single authentic case of carcinoma in a newborn infant. Statistical evidence suggests that cancerogenic agents inciting sarcoma formation act more quickly than those responsible for the formation of carcinoma, since sarcoma appears so often in early life.[5] In experimental animals, however, approximately the same length of time is required to produce sarcoma as carcinoma.

SUMMARY

A case of congenital fibrosarcoma has been reported, the infant living 22 days and dying with widespread metastasis, complicated by acute interstitial pneumonitis.

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MESOTHELIOMA OF THE PLEURA

Report of a Case Characterized by the Formation of Macrophages and Giant Cells

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DURHAM, N. C.

PRIMARY tumors of the pleura are unusual. They vary, one from another, to such an extent that classification has been exceedingly difficult. In the earlier literature the morphological characteristics led authors to designate these tumors as carcinoma.[1] Later, because of differences in appearance and behavior, their origin was described as being the endothelial lining of the pleura.[2] Still other authors, owing to the variety of cell types comprising these tumors, have interpreted them as arising from multipotential mesenchymal cells.[3] Zeckwer,[3a] for example, considered it ill advised to separate them into epithelial or endothelial categories and advocated the use of the term "mesothelioma," which had been introduced by Adami.

The presence of giant cells in mesothelioma has been described by several authors,[4] and the ability of serosal mesothelium to form giant cells is well known.[5] The case to be reported here presented such bizarre and unusual giant cells that it is thought publication is warranted.

REPORT OF A CASE

History.—The patient was a 53 year old white woman whose illness began with pain in the epigastrium approximately three months before admission. This pain was described as constant, sharp and boring in character with radiation through a short distance along the left costal margin. There were no other symptoms. On admission the patient appeared slightly undernourished but not in acute distress. The only physical finding of note was tenderness in the epigastrium and left upper quadrant of the abdomen. Hemoglobin was 70 per cent; red blood cells, 3,660,000; the corrected erythocyte sedimentation rate, 43. Other laboratory examinations, including blood chemistry, agglutination tests and skin sensitivity tests gave normal results. Roentgen examinations of the chest and abdomen showed only calcification in the

From the Department of Pathology, Duke University School of Medicine.

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4. Saccone and Coblenz.[2c] Zeckwer.[3a] Geschickter.[3c] Klemperer and Rabin.[3d]

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spleen. Throughout the patient's course in the hospital the pain persisted and her temperature fluctuated between 37 and 39 C. (98.6 and 102.2 F.), not responding to penicillin therapy. She was allowed to return to her home, where she remained in bed until she was readmitted about two weeks later. The pain persisted throughout this interval. On second admission roentgen studies of the chest indicated a definite area of increased density at the left base, which was interpreted as a pleural effusion. Bronchoscopic studies indicated a slight compression of the

Fig. 1.—A transverse section through the left side of the thorax to show the greatest volume of hemorrhagic and necrotic tumor at *A* with growth extending above the diaphragm to surround the pericardium at *B*.

left stem bronchus. The thorax was explored, and a tumor was found extending from the mediastinum laterally within the thoracic cavity. The tumor was moderately firm in consistency, yellowish red and apparently encapsulated. Histological study showed an anaplastic tumor

characterized by bizarre multinucleated giant cells with decided variation in their histological appearance but with no conformity to an established pattern. Within the following two months the patient received 1,080 roentgens of roentgen radiation therapy. Despite all therapeutic measures, she grew progressively weaker. Effusions in the left side of the chest required repeated thoracenteses. Anemia appeared, with hemoglobin as low as 9.1 Gm. (51 per cent), which responded to transfusions. Late in the course of her illness, gastrointestinal symptoms developed in the form of anorexia, nausea and vomiting. These contributed to her state of malnutrition, and she died eight months after the onset of her illness.

Autopsy (performed about 14 hours after death).—The body was malnourished, measuring 141 cm. and weighing only 90 pounds (41 Kg.). There was diffuse dark brown pigmentation over the left anterior portion of the chest, and a recent well healed incisional wound extended along the ventral half of the seventh interspace. As soon as the thoracic cavity was entered, a large mass of soft, pale, necrotic tumor tissue was encountered. This tissue was adherent to the inferior half of the sternum and required sharp dissection during the removal of the sternum. It extended along the dorsal and ventral walls of the left side of the thorax in the region of the seventh and eighth ribs and also over the left dome of the diaphragm (fig. 1). The tumor was very soft and quite friable. There was extensive necrosis, and large areas of yellowish material were found scattered throughout the tumor. In the pericardial cavity was a considerable amount of fibrinous exudate. The thickness of this exudate was approximately 3 mm., and in the dorsal wall of the pericardium was a small circular area about 3 cm. in diameter in which the tumor had eroded through the pericardium. The myocardium and endocardium showed nothing remarkable. The left lung was covered by tumor over a large portion of its inferior lobe. The tumor served to immobilize the diaphragmatic pleural surfaces throughout. The parenchyma of both lungs showed little except edema and congestion, with slight consolidation in the lower portion of the left lung.

Numerous small nodules were found scattered over the peritoneal surface of the abdominal cavity. These nodules varied in size from 2 to 15 mm. in diameter. They consisted of yellowish, soft, fleshy tissue and were similar to the mass in the thorax. These nodules were also found throughout the omental surfaces and in the pelvis, especially on the surfaces of both ovaries, where the tumor implants were quite large. The superior portion of the left lobe of the liver was joined to the inferior surface of the diaphragm by tumor. The liver weighed 1,240 Gm., and in addition to the tumor implanted on its superior surface, several nodules of metastatic growth of yellowish, soft tissue were found scattered throughout its parenchyma. The largest of these nodules was about 3 cm. in diameter. The parenchyma of the liver showed extensive chronic passive congestion, and scattered throughout were small calcified nodules. The gallbladder and the biliary tracts were not remarkable.

The gastrointestinal tract showed no abnormalities except that tumor implants were found in the appendices epiploicae. Tumor implants were also found on both ovaries. The larger, on the right, measured 25 mm. in diameter. The spleen contained numerous firm nodules about 2 mm. in diameter. Other organs of the abdomen showed no abnormalities. The vessels generally were extensively involved in an atheromatous process.

Microscopic Examination.—Various tumor areas studied by histological methods showed extreme variability. Vast areas of coagulation necrosis predominated. In the central portions of the tumor were preserved islands of viable tumor tissue. In these, as well as in the peripheral viable tumor, there was a bewildering cytologic array. Intermingled were cells of different form (fig. 2*A*). There were small, rounded cells with greatly hyperchromatic nuclei, many of which were in mitosis. With these were found spindle-shaped cells, also with hyperchromatism of their nuclei. The outstanding feature of the tumor, however, was the great number and variability of the giant cells. Some were relatively small, but the greater number were very large. The number of nuclei varied from 1 to 40 (fig. 3*A*). In some of the cells these nuclei were peripherally placed as in the Langhans type of giant cell, and in others there was no specific distribution of the nuclei. The nuclei in cells interpreted as healthy were invariably hyperchromatic. The chromatin appeared gathered into many fine, intensely stained granules. The nuclei possessed nucleoli which appeared eosinophilic. The size of the nucleolus varied in

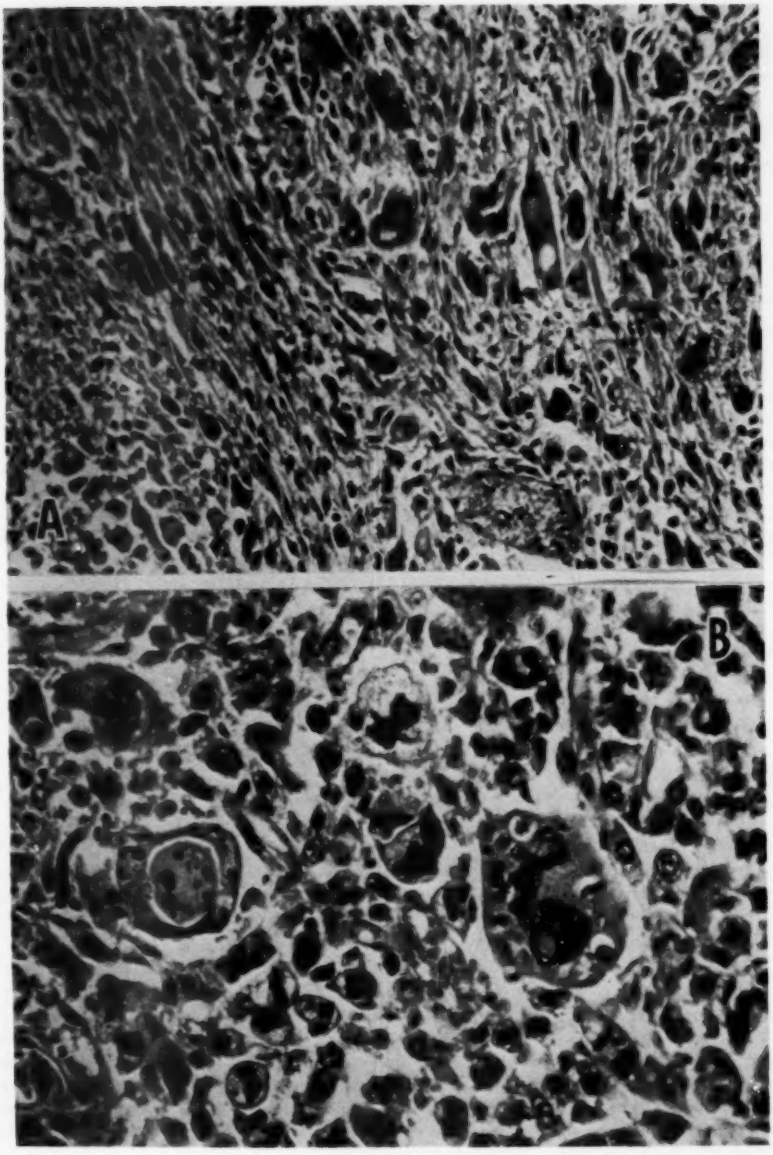

Fig. 2.—*A*, low power view showing a combination of spindle-shaped cells and giant cells. Hematoxylin and eosin; × 175.5. *B*, high power field showing the phagocytic character of the cells. The large cell on the right contains erythrocytes and leukocytes, while the giant cell on the left contains another giant cell in its cytoplasm. The intranuclear pale bodies are eosinophilic. Hematoxylin and eosin; × 966.5.

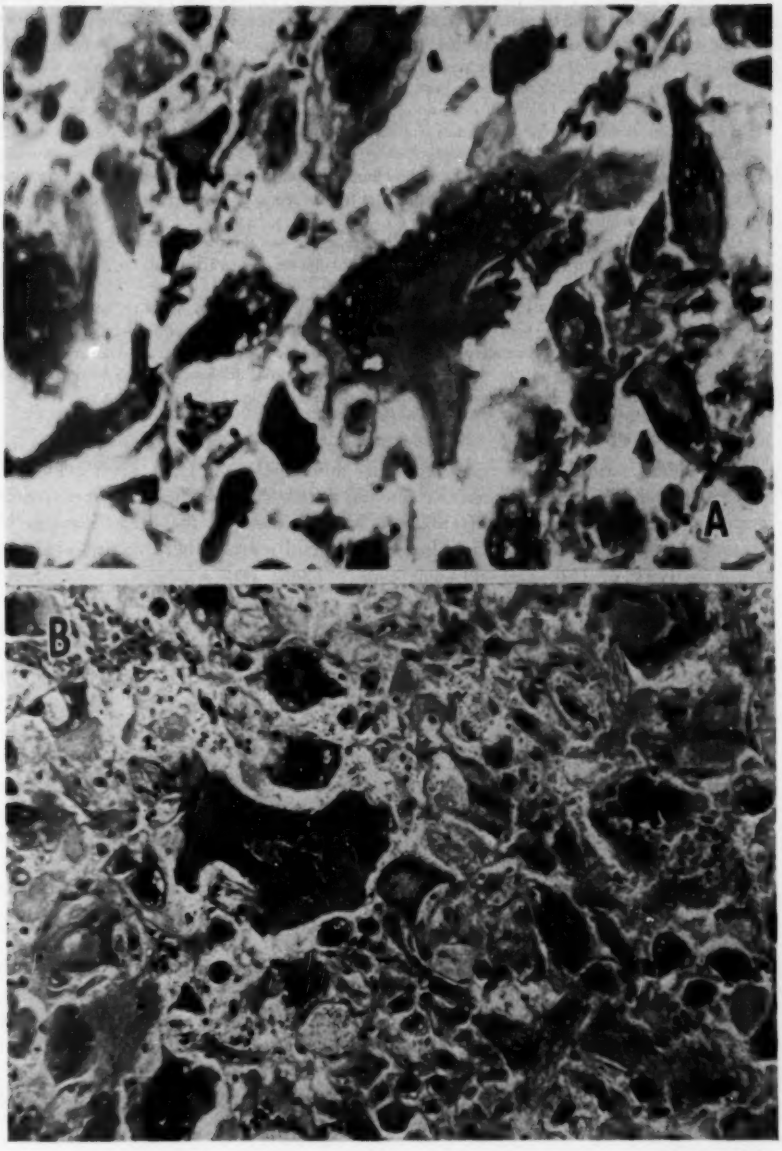

Fig. 3.—*A*, another high power field showing the unusual and pleomorphic character of the giant cells. Hematoxylin and eosin; × 966.5. *B*, a field bordering on an area of necrosis to show the disintegration of nuclei. Hematoxylin and eosin; × 966.5.

proportion to the size of the nucleus. Most of the nuclei had only one nucleolus; however, a few contained several, and as many as eight were observed in one nucleus. Nuclei were observed showing various degrees of karyorrhexis and karyolysis (fig. 3 *B*).

The cytoplasm of these cells appeared as stippled, palely stained material containing vacuoles and fat droplets. There were numerous granules of pigment, ingested red blood cells, leukocytes and even other giant cells (fig. 2 *B*). Some of these cells exhibited palely stained central zones.

In studies utilizing silver impregnation one was able to observe a delicate reticulum intimately associated with these cells. With the Masson trichrome technic these cells stained uniformly green, but the nucleoli and the granules in the cytoplasm stained intensely red. Striations were not found by any of the technics used, including the frozen section. Bacterial stains and acid-fast technics were noncontributory.

The same type of tumor cells were found incorporated within the fibrinous exudate of the pericardium, in metastatic foci in the liver and in implants on the ovaries and on the appendices epiploicae of the colon.

Histological study of many tissues (heart, lungs, pancreas, kidneys, bladder, uterus, breast, bone marrow, adrenal and thyroid) failed to show tumor. The nodules in the spleen were fibrous scars, probably indicating a healed granulomatous inflammation, possibly tuberculosis. Other conditions noted were pulmonary edema and bronchopneumonia, dilatation of the heart, chronic passive congestion of the liver, severe arteriosclerosis and Meckel's diverticulum.

COMMENT

The identification of this tumor presents difficulty. The large giant cells suggest the possibility of a chorionic carcinoma, perhaps developing in a mediastinal teratoma. This diagnosis seems unlikely because none of the other features of teratomatous growth could be identified after careful search; there was no evidence of the activity of chorionic gonadotropins. There was widespread undoubted phagocytic activity on the part of the smaller cells, as well as on that of the giant cells, of the tumor. Support for identifying this tumor with the group of spindle and giant cell sarcomas described by Symmers [6] as arising in the fat bodies of Inglis [7] is found in the presence of sudanophilic globules in the cytoplasm of the cells, but the great number of multinucleated giant cells and the location and growth of the tumor of this report seem to exclude it from this group. The separation of some tumors from certain reticuloendothelioses can be exceedingly difficult.[8] However, the growth and metastatic behavior of the tumor in this case are those of a genuine neoplasm and not a controlled proliferation. This tumor is interpreted as belonging to the group of mesotheliomas of the pleura because extreme variation in the potentials of the mesenchymal cells of the pleura is well recognized, the site of greatest mass is in the left side of the thorax, its growth is diffuse, its cytologic composition is pleomorphic, and its cells exhibit the function of phagocytosis.

The attempt to identify this case with any specific reported case is difficult because of the known variability in composition of primary tumors of the pleura. Comparison of reported cases seems to indicate that tumors of the pleura may differentiate into structures morphologically suggestive of either epidermal or mesodermal origin. While cases have been reported in which the formation of giant cells is a feature, no report of a case with such striking differentiation into macrophages and giant cells has been found.

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Klemperer and Rabin[3d] have divided primary tumors of the pleura into localized and diffuse types. They consider that the localized tumors arise from the subpleural areolar tissue under the visceral pleura and that the diffuse types develop from the surface lining cells, which have varied potentials. That the formation of giant cells may be a function of the mesenchymal cells lining the pleura seems to be reasonably well established on the basis of the activity of these cells in instances of infection, in previously reported neoplasms and in tissue culture.[3e] In the present instance the lesion behaves like a cancer in that it spreads by recognized metastatic channels but is predominantly composed of cells which exhibit the ability to function in the same way as macrophages and giant cells derived from macrophages. The ability of cells of this tumor to ingest particulate matter, specifically erythrocytes, leukocytes and other tumor cells, would place it as a "functioning tumor" derived from cells with multiple potencies, at least one of which, phagocytosis, is that of reticuloendothelial tissue.

SUMMARY

A mesothelioma of the pleura characterized by cells differentiating into functioning macrophages and giant cells is reported. This case serves to emphasize the multipotential character of tumors derived from the lining cells of the pleura in that such widespread production of cells exhibiting the property of phagocytosis has not been encountered in previously reported tumors of this derivation.

CONCURRENT LEIOMYOSARCOMA AND SQUAMOUS CARCINOMA OF THE ESOPHAGUS

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OVER 90 per cent of cancers of the esophagus are squamous carcinoma.[1] The most frequent sites of occurrence are the region of the cricoid cartilage, the bifurcation of the trachea and the cardioesophageal junction.[2] There is a high incidence in the male sex, reported by some authors as high as 87 per cent.[1]

Other cancers arising in the esophagus include adenocarcinoma, adenoacanthoma, so-called carcinosarcoma, fibrosarcoma, leiomyosarcoma and other types of sarcoma. The tumors of mesenchymal origin are found most frequently in the distal part of the esophagus and may in their growth extend either into the lumen or beyond the wall into the mediastinum. Studies of multiple tumors and cancers of the esophagus have been reported by Janeway and Green,[2] Jackson,[3] Vinson,[4] Resano and Albanese,[5] Adams,[6] Watson,[7] Pennes,[8] Pearlman,[9] Warren and Gates,[10] Owen [11] and Major.[12] These studies, representing reviews of approximately 5,000 cases, did not record two separate primary cancers, one of epithelial origin and the other of mesenchymal origin. Jackson [3] reported two cases in which there were separate carcinomas believed to be separate primary lesions, but both of those cases are open to question because of the proximity and similarity of the two types of tumor.

The case of squamous carcinoma of the middle third of the esophagus and leiomyosarcoma of the cardioesophageal junction described here is reported because it is believed to be the first recognized case of two primary cancers of the esophagus, one of epithelial origin and one of mesenchymal origin, developing simultaneously.

From the Department of Pathology of the M. D. Anderson Hospital for Cancer Research and the Department of Pathology of the University of Texas Post-Graduate School of Medicine.

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CLINICAL HISTORY

A 56 year old woman was admitted to the M. D. Anderson Hospital for Cancer Research with a history of having had difficulty in swallowing for the past eight months. Her symptoms began with inability to swallow large particles of food and progressed until she could not swallow any solid food, only liquids passing. Eleven months before admission a gastrointestinal series of roentgenograms done in another hospital revealed only a "spastic colon." She had lost 16 pounds (7 Kg.) during the past eight month period.

Family History.—The family history was irrelevant except for the fact that one of her mother's brothers had a carcinoma of the lip and another brother had a carcinoma of the face.

Physical Examination.—The complete physical examination revealed a rather thin but well developed middle-aged woman with nothing contributory to the present illness.

Laboratory Examinations.—Laboratory studies including complete blood counts, urinalysis, Kahn and Klein tests, blood nonprotein nitrogen, blood sugar, blood chlorides, blood cholesterol, total serum proteins with albumin and globulin fractions revealed normal values.

Roentgenographic Examination and Barium Sulfate Meal Studies.—This demonstrated a constricting lesion in the esophagus at the level of the pulmonary vessels. It was 6 cm. in length. The barium sulfate passage was delayed at this point.

Esophagoscopy.—With the patient under local anesthesia, esophagoscopic examination disclosed that at a distance of 32 cm. below the upper incisors a rigid constricting lesion was encountered. A biopsy specimen taken from this lesion was reported as squamous carcinoma, grade 3.

Hospital Course and Treatment.—The patient was hospitalized and prepared for surgery in the routine manner. Surgical removal of the growth was done on the fourteenth day after admission through a left thoracotomy with subperiosteal resection of the eighth rib. The esophagus was freed, and the tumor was palpated lying just beneath the arch of the aorta. No metastases were found. The diaphragm was traversed and the stomach mobilized and drawn into the chest. After mobilization, the esophagus was resected from the cardia to what was regarded as a safe distance above the tumor and the stomach was closed. An anastomosis was made between the distal end of the esophagus and the newly made circular opening in the anterior stomach wall. The stomach was sutured into position, after which the diaphragm was closed loosely about the stomach. Routine closure without drainage was carried out after reexpansion of the lung.

Postoperatively the patient's course was relatively uneventful, and she left the hospital ambulatory on her twenty-eighth postoperative day.

PATHOLOGIC EXAMINATION OF OPERATIVE SPECIMEN

The specimen consisted of an esophagus 10 cm. in length and 3 cm. in diameter (fig. 1). Rough fibrous tissue containing numerous veins filled with clotted blood composed the surface of the specimen. When opened, there was found, replacing the mucosal surface in what was the middle one third of the esophagus, a constricting ulcerated lesion measuring 4 cm. in length and extending to within 1 cm. of the upper resected edge (fig. 1 *A*). This lesion was sharply demarcated from the surrounding normal tissue by a raised edge. The ulcerated surface was raised approximately 1 cm. above the surrounding mucosa and was a mottled gray-brown in color. On cut section the tumor was a homogeneous light gray-white and extended through the muscularis in one area, but in no place did the tumor reach the outer surface of the specimen. Areas of recent hemorrhage and dilated veins were found adjacent to the lesion. A polypoid lesion measuring 1.5 by 2 by 1 cm. was noted in the lower end of the specimen (fig. 1 *B*). The surface was smooth, gray-brown, and covered by mucosa in continuous phase with the mucosa of the base. On section the mass was moderately firm and a light homogeneous gray-white in color. It extended through the normal mucosal level to the muscularis, where it

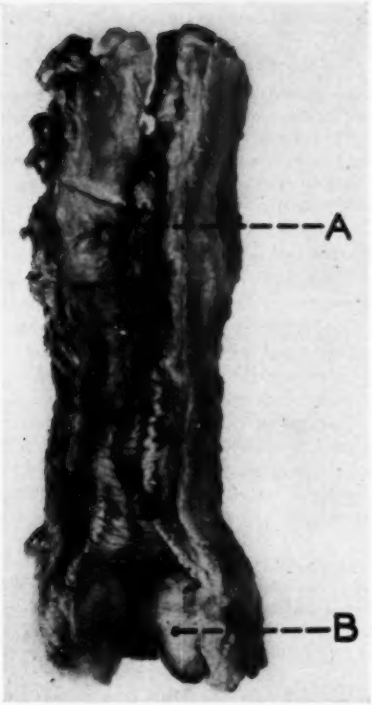

Fig. 1.—Gross surgical specimen showing two tumors: *A*, squamous carcinoma encircling the lumen and showing a slightly raised granular surface; *B*, leiomyosarcoma projecting into the lumen and attached by a pedicle.

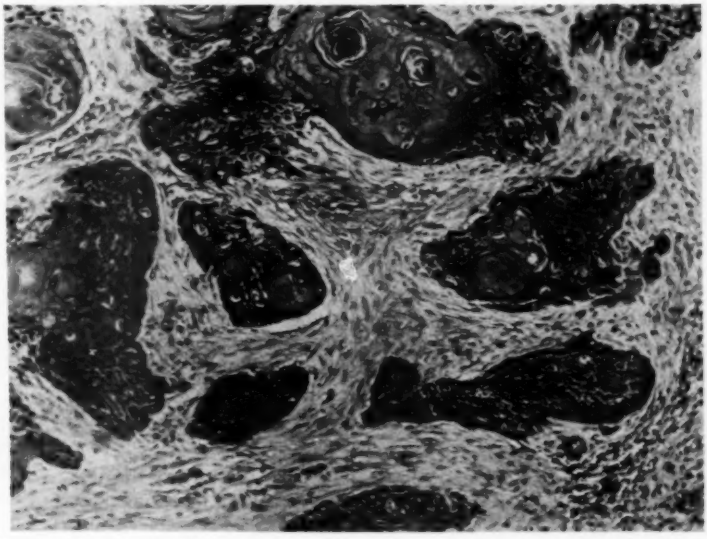

Fig. 2.—Squamous carcinoma in abundant connective tissue stroma. The squamous cells show high differentiation with pearl formations.

was diffusely attached. The pedicle attachment arose 1 cm. from the resected cardiac end and the distance between it and the previously described ulcerated lesion was 3 cm. Careful dissection of the adventitial tissue surrounding the esophagus yielded nine lymph nodes. Six lymph nodes were submitted separately with labels indicating (1) hilar region, (2) the region under the arch of the aorta, (3) a pulmonary artery and (4) adventitial nodes. Anthracotic pigmentation of varying degrees, but no grossly demonstrable tumor, was found in the nodes.

A second specimen was identified as a piece of rib 1.5 cm. in length showing no pathologic change.

MICROSCOPIC EXAMINATION

Sections taken from the lesion in the middle third of the esophagus showed masses of squamous epithelial tumor replacing the mucosa and extending into the muscularis. Cornification of the cells to a remarkable degree was noted in many

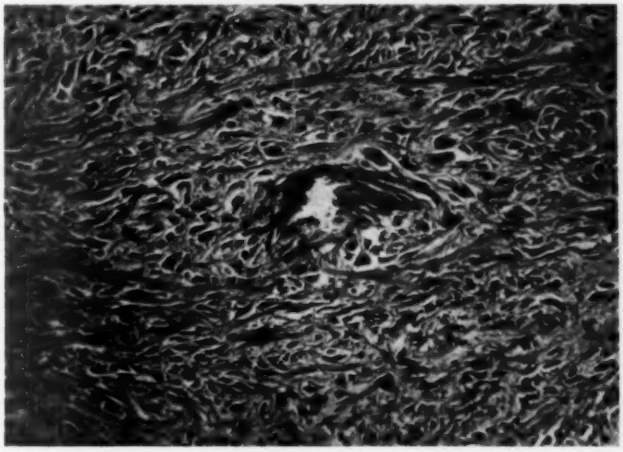

Fig. 3.—Leiomyosarcoma. The spindle-shaped cells are arranged in parallel formations.

of the masses with the formation of large oval collections of eosinophilic-staining hyaline substance (fig. 2). The cells at the peripheral parts of the masses of tumor generally were less differentiated, although frequently they formed palisading arrangements, and always stained hyperchromatically. Occasional large neoplastic giant cells were seen. The general arrangement of the tumor followed the form of the replaced mucosa. Masses of tumor were found in the muscularis, but in no instance did the tumor extend into the adventitial tissues.

Sections taken from the pedunculated tumor mass attached to the lower end of the esophagus showed gastric mucosa on one side and stratified squamous epithelium on the other. The tumor was composed of spindle-shaped or slightly elongated cells showing a moderately cellular type of growth (fig. 3). In many parts there was a collagenous intercellular substance. Many large neoplastic giant cells with atypical nuclei and atypical mitotic figures were present. The spindle-shaped cells showed their greatest growth activity at the muscularis attachment.

The cells extended between the muscle bundles of the esophageal wall for only a short distance. The surface of the tumor was not covered by epithelium but showed a narrow zone of necrosis heavily infiltrated with polymorphonuclear granulocytes. On this side of the mass there was unusual vascularity of the stroma.

None of the lymph nodes recovered from the specimen contained tumor.

FOLLOW-UP

The patient, seen every two to three months, has shown progressive improvement. There has been a 16 pound (7 Kg.) gain in weight since surgical intervention, and there have been no clinical signs of recurrence. The most recent roentgenograms and barium sulfate studies, taken 25 months postoperatively, showed slight residual deformity from the operative procedure but no evidence of metastasis or parenchymal disease. A barium sulfate swallow disclosed a normal stoma.

COMMENT

There were two cancers in the esophagus, one a typical squamous carcinoma of the middle third of the esophagus and the other a leiomyosarcoma of the lower end of the esophagus at the junction of the cardioesophageal mucosa. The squamous carcinoma was a well differentiated tumor, and in spite of extensive ulceration and replacement of the mucosa it had not extended through the muscularis.

The leiomyosarcoma histologically was a more malignant tumor, with more anaplasia of cells and numerous neoplastic giant cells. In spite of this, it likewise was confined in its distribution at the primary site to the muscularis. It is most interesting that no regional lymph node metastases were observed from either tumor. Such strikingly different histologic types leave no question as to the fact that each represents a separate primary cancer.

SUMMARY

A case of a squamous carcinoma of the middle third of the esophagus and a leiomyosarcoma of the lower end of the esophagus arising at the cardioesophageal junction is reported. This is believed to be the first reported case of two primary cancers of the esophagus arising from different germ layers.

2310 Baldwin Street.

Books Received

BAKTERIOLOGIE UND SEROLOGIE: AUSGEWÄHLTE UNTERSUCHUNGSMETHODEN FÜR DAS BAKTERIOLOGISCHE UND SEROLOGISCHE LABORATORIUM. By Dr. Lothar Hallman, Leiter der bakteriologischen und serologischen Abteilung am allg. Krankenhaus Heidberg, Hamburg-Langenhorn. Pp. 646, with 374 illustrations. Price $7. Georg Thieme Verlag, Diemershaldenstrasse 47 (14a), Stuttgart-O, 1950.

Dr. Hallman has made a laudable attempt to keep pace with the rapid advances in bacteriological and serological techniques of the past few years. He has attempted not to be compendious or complete but to present a practical handbook for workers in the field. The volume, nevertheless, runs to 646 pages; it is well illustrated and well printed throughout.

After a brief introduction covering general morphology, physiology, normal flora of the human body, and general methodology, each major group of bacteria is treated in detail. The classification follows the 1939 edition of Bergey. Methods of isolation and identification are well covered. The portion dealing with the enteric bacilli is excellent, although many of the mediums described are unfamiliar to the average American bacteriologist. More emphasis is placed on carbohydrate fermentations for the differentiation of the Salmonellae than is the practice in this country. The section covering the Clostridia appears to be particularly well done.

The section on the Rickettsiae covers only five pages and is so brief as to be practically valueless. The same, to a somewhat lesser degree, can be said of the portions dealing with viruses, the Protozoa and mycology. In the latter, Histoplasma is not even mentioned, nor is Toxoplasma in the section on Protozoa. There are occasional errors, like listing Listerella monocytogenes as the cause of infectious mononucleosis.

Serology is treated unevenly. The complement fixation reaction is given considerable space. Little attention is paid to explanations or theory.

The rest of the volume covers miscellaneous laboratory techniques and devices. Antibiotics, which are of increasing importance to the bacteriologist, are given only cursory attention, and although the book is dated 1950, only penicillin and streptomycin are mentioned.

At the end of the book is a list of formulas and stains, mediums and reagents printed on yellow paper for easy reference, which to the reviewer is an excellent idea for a book of this type.

Within the limits set out for itself, "Bakteriologie und Serologie" should be of value in continental laboratories, but the unfamilar techniques and the grossly inadequate coverage of so many important subjects seem to make this book of little value in this country, except as a source of information on the methods used in this field in Germany.

EXOTISCHE KRANKHEITEN UND KRANKHEITSVERLÄUFE. By Dr. Med. F. O. Höring, Apl. Prof. für Innere und Tropenmedizin an der Universität Tübingen, Chefarzt der inneren Klinik des Stadtkrankenhauses Worms a. Rh. Pp. 407, with 51 illustrations. Price $8.55. Georg Thieme Verlag, Diemershaldenstrasse 47 (14a) Stuttgart-O, 1950.

PATHOLOGY OF THE CENTRAL NERVOUS SYSTEM (A STUDY BASED UPON A SURVEY OF LESIONS FOUND IN A SERIES OF FORTY THOUSAND AUTOPSIES). By Cyril B. Courville, M.D., professor of nervous diseases, College of Medical Evangelists; director, Cajal Laboratory of Neuropathology, Los Angeles County Hospital, Los Angeles. Third edition. Pp. 473, with 208 illustrations. Price $9.50. Pacific Press Publishing Association, Mountain View, Calif., 1950.

The appearance of a third edition of this textbook of neuropathology is an indication that it meets a substantial need. Clear, lucid, well organized, and with format of high quality, it is of particular interest to students and clinicians, presenting in concise fashion the various data of brain pathology. The chapter on head injuries is especially interesting. The general point of view seems much more closely allied to that of the clinicians than to that of the general pathologist. Of the 208 illustrations in the text, only 35 are photomicrographs, the remainder being photographs and drawings.

Two adverse points should be noted: The title is misleading, for, of the 40,000 autopsies mentioned, only some 55 per cent included examination of the nervous system, and only in some 4,000 of the cases represented in this percentage were the specimens studied personally by Professor Courville. The bibliography leaves much to be desired and exhibits rather marked parochialism. Of a total of some 500 numbered references, about 20 per cent are the author's own previous papers, while a substantial number of others are articles published in the *Bulletin of the Los Angeles Neurological Society*. Considering, for example, that the whole subject of multiple sclerosis has been accorded only three references, one of which is an article in the Los Angeles *Bulletin*, the bibliographic policy deprives the student of many better known, more readily accessible references.

CURRENT THERAPY 1951: LATEST APPROVED METHODS OF TREATMENT FOR THE PRACTICING PHYSICIAN. Edited by Howard F. Conn, M.D. Pp. 699. Price $10. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5; 7 Grape St., Shaftesbury Ave., London, W.C.2, 1951.

This volume was prepared by the editor, 12 consulting editors and 275 contributors. The roster of names contains many tht are well known. This big book (8 by 10¾ inches [20 by 27 cm.]) has large clear type and well arranged pages. It is competently indexed in 34 triple column pages. The text is divided into 16 sections. The first is concerned with infectious diseases; following ones, with the common diseases of the various bodily systems. Some tropical and rare diseases are included, but on the whole the book is to be used in temperate climates. It deals with treatments only, not diagnosis. Present methods of therapy are given in the words of the authors, including brief but exact details of management.

The editor states that since the preparation of the 1950 edition, 49 contributors and 86 new methods have been added and many other significant changes made.

Section 15 is devoted to diseases due to physical and chemical agents.

Section 16 contains appendixes and an index. A roster of about 275 drugs gives additional data concerning doses, methods of administration, types of preparations and the names of the manufacturers. The use of the pituitary adrenocorticotropic hormone (ACTH) is discussed as observed in 15 different conditions. Under some headings, two or more methods are outlined, with no indications of preference.

This is a carefully prepared, handy volume containing descriptions of the newer methods and techniques suitable for daily use by the general practitioner.

PARASITIC INFECTIONS IN MAN. A SYMPOSIUM HELD AT THE NEW YORK ACADEMY OF MEDICINE, MARCH 15 AND 16, 1949. Edited by Harry Most. Pp. 229. Price $4.50. Columbia University Press, 2960 Broadway, New York 27, 1951.

This book consists of 14 papers. The topics cover the major problems in the worldwide distribution of parasitic infection. Each section has been prepared by an expert parasitologist or immunologist. Ten chapters present facts and theories and apply them to the possible prevention and control of parasitic infections. The physiology, biochemistry and metabolism of parasites are emphasized and related to host environment. Stress is given also to the public health aspects of prevention and eradication. Attempts are made to correlate some of the newer knowledge with established facts.

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1. SHAPIRO, S., WEINER, M., ET AL.: AM. HEART J. 40:766 (NOV.) 1950.

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